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VARIETAL DIFFERENCES IN THE PHOSPHORUS FEEDING CAPACITY OF PLANTS

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(WITH TWO FIGURES)

Introduction

Agronomists have frequently noted that different strains of common crop plants often show marked developmental contrasts when grown under uniform nutritive conditions, a tendency often termed differential feeding capacity. Such physiological differences are frequently great enough to necessitate profound modifications in tillage and fertilizer practice whenever contrasted strains of a single species are cultivated.

An experiment was undertaken to isolate sharply contrasted strains of inbred corn selected on a performance basis under controlled nutritional conditions, and next to determine which inorganic elements were ineffectually utilized.

The application of nutrients on a rational basis not only requires that the absorbing power of individual species for particular elements be known but that the optimum time for making these elements available to the roots of the growing plant should be considered. There is much discussion in the literature of plant nutrition (**12, 22, 29**) with regard to this time element, and some divergence of opinion. A further purpose of this experiment was the investigation of the problem of timed application of fertilizers, particularly with reference to the absorption of phosphates, and, finally, to vary the time of application of other inorganic elements for comparison with each other and with phosphorus.

Methods

Twenty-one inbred varieties and hybrids of Reid's Yellow Dent corn, which had been closely inbred for several years, were grown. Every variety was assigned a code number using the capital letters A to U

inclusive. The lines selected for special consideration were H, O, J, N, P, and U, which, according to the Ames code, were lines *Sto* and *Wal,* and the hybrids *Ost* × *Wal, Wal* × *Ost, Wal* × *Idt,* and *Ldg* × *Sto* respectively. The explanation of the nomenclature used at the Iowa State College, Ames, Iowa, for these standard, first-cycle, inbred lines of Yellow Dent is as follows:

Sto, stocky plant type with high row number (20–24 rows).
Idt, a stiff-stalked imbred line from Iodent variety.
Wal, from Walden's strain of Reid's Yellow Dent, characterized by the fact that the silks emerge before the anthers dehisce.
Ldg, abbreviation for Lancaster Dark Green, a striking inbred line (our best) from Lancaster Sure Crop variety.
Ost, from Osterland's strain of Reid's Yellow Dent.

TABLE I

NUTRIENT SOLUTIONS
(WEIGHT OF SALT IN GRAMS, DISSOLVED IN 14 LITERS OF WATER)

MINUS POTASSIUM (− K)		MINUS CALCIUM (− Ca)		MINUS NITROGEN (− N)		MINUS PHOSPHORUS (− P)	
$NaNO_3$	1.68	KNO_3	2.00	KCl	1.48	KNO_3	2.00
NaH_2PO_4	1.80	KH_2PO_4	2.00	KH_2PO_4	2.00	$MgSO_4$	2.00
$MgSO_4$	2.00	$MgSO_4$	2.00	$MgSO_4$	2.00	$Ca(NO_3)_2$	8.00
$Ca(NO_3)_2$	8.00	$NaNO_3$	8.32	$CaCl_2$	5.50		

EIGHT POTASSIUM (8 K)		EIGHT CALCIUM (8 Ca)		EIGHT NITROGEN (8 N)		EIGHT PHOSPHORUS (8 P)	
KNO_3	4.00	KNO_3	2.00	KNO_3	2.00	KNO_3	2.00
KH_2PO_4	4.00	KH_2PO_4	2.00	KH_2PO_4	2.00	KH_2PO_4	16.00
$MgSO_4$	2.00	$MgSO_4$	2.00	$MgSO_4$	4.00	$MgSO_4$	2.00
$Ca(NO_3)_2$	8.00	$Ca(NO_3)_2$	32.00	$Ca(NO_3)_2$	8.00	$Ca(NO_3)_2$	2.00
K_2SO_4	9.10	$CaCl_2$	12.64	NH_4NO_3	32.80		
KCl	5.74						

UNIT PHOSPHATE (KNOP'S)		QUARTER PHOSPHATE* (0.25 P)		TENTH PHOSPHATE* (0.1 P)		FIVE-HUNDREDTHS PHOSPHATE* (0.05 P)	
KNO_3	2.00	KNO_3	2.24	KNO_3	2.29	KNO_3	2.31
$MgSO_4$	2.00	$MgSO_4$	2.24	$MgSO_4$	2.29	$MgSO_4$	2.31
$Ca(NO_3)_2$	8.00	$Ca(NO_3)_2$	8.96	$Ca(NO_3)_2$	9.16	$Ca(NO_3)_2$	9.24
KH_2PO_4	2.00	KH_2PO_4	0.56	KH_2PO_4	0.23	KH_2PO_4	0.08

* Quarter phosphate designates one-fourth the amount of phosphorus supplied in the unit phosphate solution; tenth and five-hundredths indicate $\frac{1}{10}$ and $\frac{5}{100}$ respectively of the phosphate used in the unit phosphate solution.

Ten seeds of each variety were planted in triplicate in three 2-gallon jars of sterilized quartz sand and later thinned to five. Twelve different nutrient solutions were used to provide the culture media for these plants (table I).

Constant temperature and humidity were maintained in the greenhouse during the growing period and an optimum amount of distilled water was added as needed. The culture solutions were made available to the plants according to a definite prearranged schedule (table II).

TABLE II

FEEDING PLAN

NUTRIENT SOLUTIONS

UNIT PHOSPHATE	QUARTER PHOSPHATE	TENTH PHOSPHATE	FIVE-HUNDREDTHS PHOSPHATE	MINUS PHOSPHATE
8000 cc. used	500 cc. used, followed by 7500 cc. of minus phosphate solution	500 cc. used, followed by 7500 cc. of minus phosphate solution	500 cc. used, followed by 7500 cc. of minus phosphate solution	8000 cc. used

SERIES	BEFORE CHANGE	AFTER CHANGE
	– K, – Ca, – N, OR – P	8 K, 8 Ca, 8 N, OR 8 P
a*	4 liters	6 liters
b*	6 liters	4 liters
c	8 liters	2 liters
d	10 liters	No change

8 K	10 liters	(Eight potassium solution)
8 Ca	10 liters	(Eight calcium solution)
8 N	10 liters	(Eight nitrogen solution)
8 P	10 liters	(Eight phosphorus solution)
0.25 P	10 liters	(Quarter phosphate solution)
0.10 P	10 liters	(Tenth phosphate solution)
Knop's	10 liters	(Knop's solution (table I)

* The pots of – K, – Ca, – N, and – P in the (a) series were carried for approximately 3 weeks on 4 liters of the – K, – Ca, – N, and – P solutions respectively, then changed to 8 K, 8 Ca, 8 N, and 8 P solutions until all pots in the series had received a total of 10 liters of nutrient solution. Similarly after using 6 liters of the minus solution on the pots of series (b), the plants were given 4 liters each of 8 K, 8 Ca, 8 N, and 8 P nutrient solutions respectively.

Eight seeds of each variety were selected at random, cracked with a pestle, dried at a constant temperature of 105° F., and ashed for phosphorus analysis.

Measurements of height were recorded 75 days after planting for varieties H, N, P, and U. Beginning 30 days after planting, the plants of certain pots of varieties H, O, and J were regularly measured, the approximate height in centimeters having been recorded every week until these plants were harvested at the age of 88 days. Sixty days after planting, these plants were photographed.

At harvest all the feeding groups of the phosphate series were selected for special study except the – P (c) series which had received the same treatment as the – P (d) series, and the – P variety O series, all the plants of which were dead. Other plants of the variety H, together with varieties N, P, and U, which had been fed with unit, quarter, tenth, five-hundredths, and minus solutions (table II) were harvested at the age of 105 days.

Plants for analysis were harvested at approximately the same time of day to minimize diurnal variations in the pH and in the carbohydrate content of the tissues. Pot contents were removed to a pan with running water, care being taken to avoid breaking the roots, then the sand was carefully washed from the roots after which they were finally rinsed with distilled water and dried.

Samples for carbohydrate analysis were comminuted, mixed, and immersed in hot 80 per cent. alcohol, tops and roots being preserved separately. These were later analyzed for reducing sugars, total sugars, and polysaccharides by a modification of the method outlined by MAHIN and CARR (**25**).

Smaller samples for inorganic elements, ash, and dry weight determinations were placed in weighing bottles and crucibles and dried in an oven at 105° F. Ash was dissolved in hydrochloric acid and made up to volume from which aliquots were used for calcium, potassium, and phosphorus determinations. Organic nitrogen was determined by the Gunning method (**4**), phosphorus by a modification of the Bell-Doisy colorimetric method (**3**), and the pH values of the freehand leaf sections were determined by SMALL'S range indicator method (**34**), non-toxic solutions of the following indicator dyes being used: bromphenol blue, bromcresol green, methyl red, bromcresol purple, bromthymol blue.

The pH values of the soil solution taken from the pots 83 days after planting were determined potentiometrically against a bubbling hydrogen electrode. Photomicrographs were made of sections of leaf, stem, and root cut 10 μ thick from material preserved in F. A. A. at harvest. Potassium chloroplatinate was converted to potassium iodoplatinate by the addition of potassium iodide and the potassium content ascertained by titrating with N/100 sodium thiosulphate delivered from a micro-burette calibrated to 0.01 cc. (**33**). Calcium was precipitated as calcium oxalate and titrated with potassium permanganate.

Discussion of results

The scheme for introducing the various feeding solutions in this experiment has been arranged in tabular form (table II). As the plants from the first varied tremendously in their rapidity of growth, the eight unit solutions were introduced into the (a) series on different dates as follows:

–K (a) H was changed Oct. 22nd and –K (b) H 19 days later
–K (a) O was changed Oct. 22nd and –K (b) O 19 days later
–K (a) J was changed Oct. 26th and –K (b) J 24 days later
–P (a) H was changed Oct. 24th and –P (b) H 21 days later
–P (a) O was changed Oct. 24th and –P (b) O 21 days later
–P (a) J was changed Oct. 31st and –P (b) J 19 days later
–N (a) H was changed Oct. 26th and –N (b) H 28 days later
–N (a) O was changed Oct. 26th and –N (b) O 28 days later
–N (a) J was changed Nov. 8th and –N (b) J was not changed
–Ca (a) H was changed Oct. 18th and –Ca (b) H 20 days later
–Ca (a) O was changed Oct. 18th and –Ca (b) O 20 days later
–Ca (a) J was changed Oct. 30th and –Ca (b) J 15 days later

The time required for each variety to utilize the first 4 liters of the minus solution varied with the mineral element more than with the variety as shown by the following data:

Variety	–K	–P	–N	–Ca
	Days before a change in nutrient			
	days	*days*	*days*	*days*
H	19	21	28	20
O	19	21	28	20
J	24	19	.....	15

Varieties H and O took up water at about the same rate. Variety J took up water at a slower or faster rate than varieties H and O, depending upon the mineral lacking in the nutrient solution.

The beginning of the use of the eight unit concentration had a toxic effect on two of the (b) series causing –K (b) O to be 2 cm. shorter than –K (c) O, and –N (b) H to be shorter by the same amount than –N (c) H. When a strong concentration of an element was supplied as late as the eighth or the ninth week after planting, instead of immediately increasing the growth rate, as the same solution did to series (a) 6 weeks after planting, it had a depressing effect upon the growth rate of the plants. As an example of this reaction, –K (b) H was increasing at the rate of 2 or 3 cm. a week, but after the 8K solution was introduced about the tenth week the average increase was about 1 cm. a week thereafter. The total increase in height,

if there was any increase at all, was very slight after the introduction of the eight-unit solutions in the (b) series.

The effect of withholding a single element from each (b) series in the minus groups varied with the element omitted. The average increase in height of (a) over (b), in varieties H, O, and J, was 8 cm. in. –K, 10 cm. in – P, 4 cm. in – Ca, and 3 cm. in – N. The average time between the introduction of 8K, 8P, 8Ca, and 8N solutions in the (a) and the (b) series was 21 days for – K, 20 days for – P, 18 days for – Ca., and 28 days for – N. The effect, as measured by increase in height, was greater when either phosphorus or potassium was added rather early in the life of the plant, after having been withheld for 5 weeks or longer, than when either calcium or nitrogen was similarly withheld and later added in abundance. It has been established by many plant physiologists (**2, 8, 9, 10, 32, 38, 39**) that an adequate phosphate supply should be made available early in the life of the corn seedling. The response was generally prompt and significant when a high concentration of potassium or phosphorus was added after 6 or 7 weeks, but after 9 or 10 weeks corn plants did not respond to the introduction of the element too long delayed.

The most outstanding example of the injurious effects of withholding an element too long was –P (b) O. As soon as the 8 P solution was introduced, these plants were noticed to be dying. By the time of harvest, 16 days later, there was apparently no living tissue left in the plants of this series. The lower leaves of the plants died early, then the newer leaves began to curl and to die at the tips. After about 22 cm. in height was attained no further increase in growth was observed. Phosphorus was not available for the acceleration of the vital life processes, such as mitosis, reduction of nitrates, and respiration, so growth could not continue and deterioration of plant tissue began at once.

At frequent intervals during their growth the plants were observed for differences in general appearance. These differences became apparent as soon as the shoots of the seedlings appeared, and became more complex and accentuated thereafter. Chlorosis became noticeable very early in the unit phosphate P and U varieties. LOEHWING (**21**) showed that diminished acidity of the sap interfered increasingly with iron mobility until it finally induced chlorosis. The acidity of the leaf mesophyll of these chlorotic series, particularly that of variety P, was later found to be much lower than the acidity of most of the groups which showed no such evidence of chlorosis. Hence the pronounced chlorosis in these series confirms LOEHWING'S observations and was, in part at least, due to an iron deficiency. According to JONES (**15**), chlorosis in corn is often due to a magnesium deficiency. If the absorption of calcium nitrate may be assumed to be molecular, the utilization of this form of nitrate in sufficient amounts to produce the vegetative luxuri-

ance observed in these series would have resulted in the absorption of excess calcium which probably produced chlorosis in the leaves by rendering iron insoluble and by depressing the intake of magnesium. It has been experimentally shown by other workers that manganese deficiency probably always causes chlorosis in corn, and that this occurs more frequently than a deficiency in magnesium or iron severe enough to cause chlorosis (**9, 24**).

Many symptoms of phosphorus deficiency were noted. Red leaves were prominent on plants of unit phosphate N, and on all plants of variety U (**13, 16, 23, 36**). All other plants except unit phosphate P were observed to have small, dark green leaves. The leaves of many of the dwarfed plants had curled and died at the tips. There was much purple coloring in the roots of all series except unit phosphate P, N, and U. The absence of lateral root branches and root hairs was particularly noticeable in all the plants of the H variety. Maturation was delayed in all the plants (**13**), for reproductive activities should have been well begun long before the 105th day after planting. An increased phosphorus absorption would doubtless have abbreviated the vegetative growth period so that most tassels and silks would have appeared as early as the ninetieth day or earlier.

Among the varied evidences of deficiency and toxicity in the plants during the growth cycle there were many instances of premature death due to physiological disease (table III). As mentioned above, the – P (b and c) series of variety O all died before the first application of the 8 P solution. These plants died of phosphorus starvation. This same delay in the application of phosphates through the culture media did not prove so injurious to the other two varieties, H and J. The plants in the 8 N solutions all died early because of the excessively high transpiration rate produced by the heavy application of nitrogen. The first sign of approaching death in these plants was the appearance of marginal dead strips on the leaves, and dying at the tips.

The presence of anthocyanin pigment in the leaf in sufficient amount to cause a red color was common to nearly all the plants of variety H, regardless of the concentration of the solution, showing the phosphorus-deficient character of the strain (**13, 22**). The red was less conspicuous in – P (a) H, 8 P H, 0.25 P H, and 0.1 P H. The purple coloration of the roots in 0.1 P H was an indication of phosphorus insufficiency (**22**). The redness of the leaves on plants of the 8 K solution was due to the depression of Ca, which if present would have increased the mobility of the iron and made possible the continued formation of chlorophyll in the leaves instead of permitting the anthocyanin pigment to replace the chlorophyll in the subepidermal cells of the leaf. Red leaves appeared on – Ca (b, c, and d) series for the same reason.

TABLE III

DEFICIENCY AND OTHER SYMPTOMS

SOLUTION	VARIETY H	VARIETY O	VARIETY J
−Ca	Plants green, 11–11		Male flowers shedding pollen, 11–30
(a)	Much chlorosis in leaves, 11–30		
−Ca	Red leaves, 11–11		
(b)	Much red in leaf tips, 11–30		
−Ca	Red leaves, 11–11	Slightly shorter than (a) and (b), 11–11	Some anthocyanin streaks in leaves, 11–30
(c)	Much red; nearly all dead, 11–30		
(d)			
8 Ca	Leaf tips fired and dry; tips dead and pendent, 11–11	Definite white margins; lower leaves chlorotic; central strip green; few plants alive, 11–30	Leaf stiff, erect—not pendant, less firing at tips than in H, 11–11
	Pale yellow, 11–30		
−K	Red in margins, 11–30	Uninjured. Taller than in −K (b). On 8 K	Smaller than −K (b), 11–11
(a)		Smaller than −K (c)	
−K		Pale upper leaves. On 8 K, 11–11	
(b)			
8 K	Chlorotic. Leaf margins yellow; central veins pale green, 11–11. Much red in leaves, 11–30	Much red along tips and margins of leaves.	Greener about veins than in islets. Red margins; lower leaves dead, 11–11. Much red in margin, 11–30
		Less and less color in new leaves, 11–11	
−N	Pale yellow, 11–11	Greener than −N (b)	Taller and larger leaves than −N (b), 11–11
(a)		Changed to 8 N, 11–11	Finest plants, 11–30
		Good color, 11–30	
−N	Red line at edge of leaf, 11–11		
(b)		Smaller than in (b)	
−N		Should be same. Both still on −N, 11–11	
(c)			
(d)			
8 N	Plants all dead, 11–11	Tips and margins of leaves dry, dead and yellow, 11–11	Barely alive, 11–11
			Plants all dead, 11–30
−P	Plants all green, 11–11. No red; much chlorosis, 11–30	Taller than −P (b), 11–11. Taller than H. Some chlorosis and female flowers, 11–30	Taller than −P (b)
(a)			Whitish or yellow leaf tips, 11–11. Taller than H, 11–30
−P	Changed, 10–24	Not on 8 P, 11–11	Good color; narrow leaves, 11–30
(b)	Red leaves, 11–11	Long since dead, 11–30	Changed, 11–23
(c)	Some red in lower leaf margins; female flowers, 11–30	Changed, 11–24	
(d)			
8 P	Normal in color, 11–11. Chlorosis in 43. Red spots near tip in 45, 11–30	Normal color. No larger and not so green as Knop's, 11–11. Upper leaves yellow, 11–30	Color normal. Best 8 P plants, 11–11
			None green, 11–30
0.25 P & 0.10 P	Healthy plants, 11–11. Roots purple, 11–30	Same height as 8 P; normal in color, 11–11	Healthy plants, 11–11. 0.25 P roots purple, 0.10 P roots white and very fibrous, 11–30
		Roots white, colored slightly at tip	

White and yellowish leaves on the plants of 8 Ca were due to Ca toxicity. The chlorosis in leaves, wherever it occurred, was due to some disturbance in the normal nutritive relations and may have been the result of Fe, Mg, or Mn deficiency as suggested above.

From the dry weight measurements of the plants the top-root ratio for each series was computed (tables IV and VI). Comparing these ratios with the photographs of the plants (fig. 1) there is evidence that a high T/R (top-root) ratio is generally associated with greater abnormality in growth than a moderately low one, especially for corn that has remained unduly in the vegetative stages of development. The plants that showed the most vigorous growth were those of unit phosphate P, and since these had a T/R ratio of 1.25 (table IV) this seems to be the optimum T/R ratio for plants growing in this or in a similar environment.

A marked contrast was found in the ability of the four varieties, H, N, P, and U to remove phosphorus from the solutions. In all the lower concen-

TABLE IV

GROSS ANALYSIS OF FOUR VARIETIES
(ALL WEIGHTS EXPRESSED IN GRAMS)

SOLUTION	VARIETY	AVERAGE LIVE WEIGHT PER PLANT		AVERAGE DRY WEIGHT PER PLANT		RELATIVE MOISTURE CONTENT		PERCENTAGE OF DRY WEIGHT		TOP-ROOT RATIO
		TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT	
		gm.	*gm.*	*gm.*	*gm.*			%	%	
1.00 P	H	5.8	3.9	0.67	0.45	5.13	3.45	11.5	11.6	1.50
0.25 P	H	0.9	0.8	0.09	0.04	0.81	0.76	10.2	5.0	2.25
0.10 P	H	1.6	1.6	0.15	0.08	1.45	1.52	9.4	5.1	1.88
0.05 P	H	2.6	2.5	0.27	0.12	2.33	5.38	10.5	5.1	2.25
−P	H	1.2	1.0	0.14	0.06	1.06	0.94	11.5	6.1	2.30
1.00 P	N	26.3	11.9	3.84	0.77	22.46	11.13	14.6	6.5	5.00
0.25 P	N	7.0	12.4	0.90	0.71	6.10	11.69	13.8	5.7	1.27
0.10 P	N	6.3	9.6	0.78	0.47	5.52	9.13	12.4	4.9	1.66
0.05 P	N	3.2	8.2	0.35	4.45	2.85	7.75	10.8	5.5	0.78
−P	N	6.3	6.8	0.66	0.32	5.64	3.48	10.4	4.7	2.06
1.00 P	P	19.3	27.1	2.97	2.38	16.53	24.72	15.4	8.8	1.25
0.25 P	P	2.9	4.0	0.35	0.37	2.55	3.63	11.9	9.2	0.95
0.10 P	P	3.0	5.6	0.46	0.80	2.54	4.80	15.2	14.3	0.58
0.05 P	P	4.5	3.4	0.60	0.29	3.90	3.11	13.4	8.4	2.07
−P	P	5.5	8.6	0.82	0.61	4.68	7.99	15.0	7.1	1.34
1.00 P	U	31.2	25.2	4.13	1.97	27.37	23.23	13.1	7.8	2.10
0.25 P	U	5.7	6.5	0.69	0.47	5.01	6.03	12.1	7.3	1.46
0.10 P	U	2.8	7.1	0.35	0.50	2.55	6.60	12.4	7.0	0.70
0.05 P	U	3.1	5.7	0.42	4.43	2.68	5.27	13.5	7.6	0.98
−P	U	4.7	5.2	0.74	0.53	3.96	4.67	15.7	10.1	1.40

trations of phosphorus there was strong tendency for some varieties to develop a high internal sap acidity, which impeded normal growth and development. Consequently an inefficient root system developed, which resulted in a general dwarfing of the entire plant. The relative weights of phosphorus were determined for each top and each root. These were found to coincide generally with the size and configuration of the roots and somewhat less closely with the height and general development of the whole plant (fig. 1). GILE and SMITH (**11**), growing millet in quartz sand cultures, reported a similar observation.

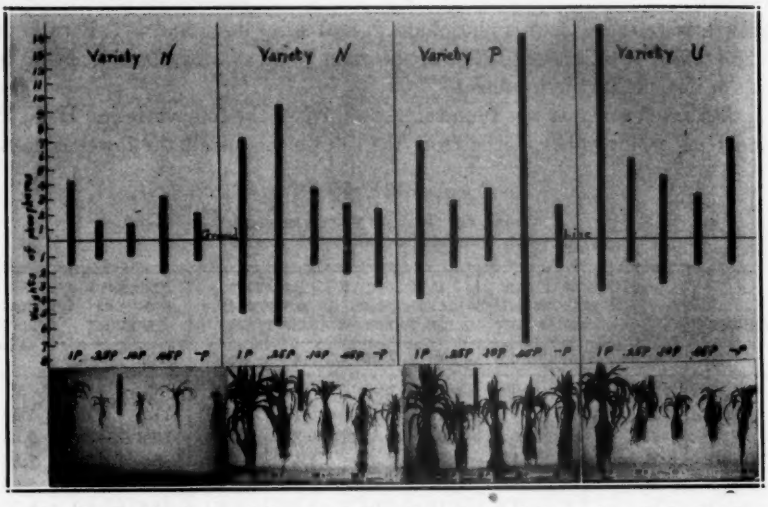

FIG. 1. Graphs for varieties shown together above photographs of plants whose phosphorus contents they represent; data from tables I and II. Phosphorus readings of unit phosphate series expressed in mg.; all other phosphorus readings in 0.1 mg. Differences in phosphorus feeding capacities of plants are shown by comparative weights of phosphorus they contained; effect of phosphorus thus absorbed on the habit of the plants are noted in photographs of the corresponding plants shown below.

It is stated by LOEHWING (**20**) that a high crop yield is associated with high organic nitrogen and high total carbohydrate content. This would probably mean a moderately low C/N ratio, but both the numerator and the denominator of the fraction would represent a measure of plant food material well above the average. MURNEEK and GILDEHAUS reporting an investigation by WALLACE say that the omission of potassium from a complete fertilizer results in an increased absorption of nitrogen and phosphorus, and when either nitrogen or phosphorus is omitted from the fertilizer there is a decreased absorption of the other two elements. In the

TABLE V

CHEMICAL ANALYSIS OF FOUR VARIETIES
(PERCENTAGES IN TERMS OF DRY WEIGHT)

VARIETIES		AV. LIVE WT. IN GM.		ASH		TOTAL NITROGEN		REDUCING SUGARS		TOTAL SUGARS		STARCH		PHOSPHORUS		C/N RATIO
		TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT	
		gm.	*gm.*	%	%	%	%	%	%	%	%	%	%	%	%	
1.00 P	H	5.8	3.9	18.2	41.0	4.27	1.89	7.6	0.3	14.6	3.3	21.3	8.0	0.226	0.210	8
0.25 P	H	0.9	0.8	16.5	20.0	3.02	2.35	2.6	6.8	5.1	12.7	5.2	7.5	0.140	0.210	3
0.10 P	H	1.6	1.6	16.3	17.3	3.37	2.38	7.2	6.0	12.5	11.4	5.4	10.7	0.076	0.048	6
0.05 P	H	2.6	2.5	17.3	18.1	3.40	3.13	3.5	0.4	5.1	0.8	7.5	14.1	0.134	0.088	5
– P	H	1.2	1.0	16.9	17.7	3.15	2.25	7.9	5.6	9.3	8.1	5.2	8.7	0.150	0.102	5
1.00 P	N	26.3	11.9	7.8	26.0	2.04	2.16	6.8	1.9	11.5	20.3	11.9	12.5	0.130	0.042	13
0.25 P	N	7.0	12.4	10.8	10.8	2.65	2.33	3.0	9.1	12.3	17.0	9.7	16.9	0.105	0.080	11
0.10 P	N	6.3	9.6	9.1	12.7	2.55	3.40	4.2	11.4	5.0	12.5	9.0	16.8	0.033	0.022	7
0.05 P	N	3.2	8.2	12.6	9.4	2.75	2.48	6.5	3.9	11.1	8.1	16.3	22.3	0.069	0.046	11
– P	N	6.3	6.8	12.8	14.2	2.71	2.16	1.7	8.9	22.7	17.0	11.7	22.9	0.039	0.095	14
1.00 P	P	19.3	27.1	7.6	18.3	2.13	1.81	16.2	15.9	19.4	16.7	11.8	11.5	0.230	0.130	15
0.25 P	P	3.0	4.0	11.9	14.3	2.61	2.07	4.0	3.5	5.0	5.1	11.2	9.1	0.058	0.044	7
0.10 P	P	3.0	5.6	9.4	13.5	2.66	2.23	2.8	1.3	8.6	5.5	12.0	7.7	0.069	0.019	7
0.05 P	P	4.5	3.4	9.2	14.2	2.35	3.70	2.4	6.6	4.9	6.7	8.9	12.5	0.216	0.028	6
– P	P	5.5	8.6	9.3	8.7	2.62	2.34	10.6	17.1	15.6	19.3	17.2	15.9	0.022	0.026	13
1.00 P	U	31.2	25.2	11.2	23.4	2.36	1.92	12.2	14.0	13.1	14.1	11.9	13.8	0.328	0.135	12
0.25 P	U	5.8	6.5	15.4	21.5	3.02	1.78	7.9	11.3	14.3	12.9	14.8	12.5	0.076	0.023	11
0.10 P	U	2.6	7.1	14.8	16.8	2.85	1.69	4.2	7.0	5.5	10.2	7.8	13.9	0.104	0.054	11
0.05 P	U	3.1	5.7	13.7	20.7	1.84	1.08	3.7	6.4	4.8	9.6	8.0	13.3	0.070	0.032	13
– P	U	4.7	5.2	14.2	18.9	2.60	1.63	3.1	3.8	4.3	6.3	7.3	8.2	0.090	0.023	6

present investigation, therefore, the omission or near omission of phosphate from the nutrient solutions readily accounts for such high C/N ratios as 13 and 14 (table V). The average C/N ratio for each variety, found by dividing the sum of the C/N ratios by the number of concentrations is given below:

Variety	Av. C/N
H	5.2
N	11.2
P	9.6
U	10.6
Average	9.2

When the curve for phosphorus content was plotted with that for the C/N ratios, they were found to be similar in many respects. Two exceptions were found. In the five-hundredths phosphate series the C/N ratio dropped to a low value while the phosphorus content rose high—variety P. In minus phosphate P the reverse situation prevailed. In the two last named concentration series the average percentages of nitrogen found were as follows:

Series	% Nitrogen
0.05 phosphate	2.53
Minus phosphate	2.48

Since these two percentages are practically the same, the great difference in the C/N ratios must depend on the carbohydrate contents. The average percentages of sugar and starch found in the same series of plants follow:

Series	Total sugars	Starch	Total
	%	%	%
0.05 phosphate	5.8	10.7	16.5
Minus phosphate	17.5	16.5	34.0

The average percentages of phosphorus for the same groups are given below:

Series	% phosphorus
0.05 phosphate	0.122
Minus phosphate	0.024

As one would expect, variety P absorbed more phosphorus from the solution with five-hundredths phosphate than from the minus phosphate solution. Although there were wide variations in the percentages of phosphorus absorbed from their culture media by the different series of plants, the amounts available had an effect upon the C/N ratios. In this experiment the better growths have usually been accompanied by high C/N ratios. Larger amounts of available phosphorus made possible a culture medium better adapted to growth with reference to acidity, led to the development of a better absorptive system, the materials for utilization in carbohydrate synthesis were available, and growth approaching normality more generally occurred.

The results of chemical analyses (table VII) show the tendency of the percentage of potassium to increase as that of phosphorus increases, an evidence that these two elements are absorbed together by corn plants. This is in agreement with the conclusions of ARRHENIUS (1). The depressing

TABLE VI

GROSS ANALYSIS OF THREE VARIETIES
(ALL WEIGHTS ARE EXPRESSED IN GRAMS)

VARIETY		AV. LIVE WEIGHT		PERCENTAGE OF DRY WEIGHT		AV. DRY WEIGHT PER PLANT			T/R RATIO	C/N RATIO	K/Ca RATIO
		TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOTAL			
		gm.	*gm.*	%	%	*gm.*	*gm.*	*gm.*			
−P(a)	H	40.0	17.9	10.0	7.4	4.00	1.32	5.32	3.0	13.2	0.09
−P(b)	H	10.8	11.3	11.8	7.9	1.27	0.88	2.15	1.5	15.0	0.14
−P(d)	H	8.6	11.1	9.4	5.9	0.81	0.65	1.46	1.3	10.8	0.16
8 P	H	63.0	29.5	8.9	8.4	5.61	2.48	8.09	2.3	17.2	0.24
Knop	H	24.8	15.0	9.4	10.5	2.33	1.58	3.91	1.5	16.5	0.15
0.25 P	H	26.6	17.3	10.8	6.5	2.87	1.12	3.99	2.5	16.9	0.11
0.10 P	H	15.8	12.5	10.3	5.4	1.63	0.68	2.31	2.4	10.3	0.12
−P(a)	O	19.9	15.0	11.5	6.8	2.99	1.02	3.31	2.2	14.5	0.08
−P(d)	O	20.1	9.7	16.5	8.5	3.32	0.82	4.14	4.0	7.2	0.10
8 P	O	46.7	21.7	12.7	8.2	5.92	1.78	7.70	3.3	6.9	0.29
Knop	O	43.2	20.0	15.9	10.5	6.87	2.10	8.97	3.3	12.2	0.09
0.25 P	O	34.0	20.0	12.4	7.5	4.22	1.50	5.72	2.8	12.3	0.14
0.10 P	O	32.3	22.3	12.1	8.3	3.91	1.85	5.76	2.1	13.4	0.11
−P(a)	J	34.8	17.8	8.2	7.2	2.85	1.28	4.13	2.2	16.6	0.12
−P(b)	J	11.8	10.1	13.1	7.1	1.55	0.72	2.27	2.1	7.4	0.23
−P(d)	J	13.2	11.1	12.4	7.9	1.64	0.88	2.52	1.9	13.0	0.06
8 P	J	48.7	20.6	12.6	8.1	6.14	1.67	7.81	3.7	16.2	0.84
Knop	J	18.4	15.3	13.1	9.4	2.40	1.44	3.84	1.7	10.4	0.12
0.25 P	J	33.4	27.0	11.4	6.6	3.81	1.78	5.59	2.1	17.4	0.07
0.10 P	J	24.0	16.0	11.7	6.8	2.81	1.09	3.90	2.6	10.2	0.16

effect of the absorption of potassium upon the absorption of calcium is also shown particularly for the 8 P series. In almost every instance, as the percentage of potassium increases in plants fed with 8 P solutions, calcium decreases. In some cases, particularly in the roots, where phosphorus and potassium both decrease in the – P (d) series, which received no phosphate, calcium is seen to decrease also, but the absorbing power of the roots is reduced to such a degree in phosphorus-starved plants that the intake of all inorganic elements is materially diminished. This fact is further demonstrated by the general decline in the percentage of ash, as the dilution of phosphate approaches zero as a limit.

Examples of plants with high phosphorus absorption percentage and which made but little growth were: one-fourth phosphate H, five-hundredths phosphate H, minus phosphate H, one-fourth phosphate N, minus phosphate N, five-hundredths phosphate P, and minus phosphate U. Examples of plants which showed a very high phosphorus absorption rate and made very vigorous growth were unit phosphate N, P, and U (fig. 1).

These seemingly contradictory facts are understandable when it is remembered that the high response plants which failed to make satisfactory growth were all from series which had been fed with the lower concentrations of phosphate, the majority being from five-hundredths and minus phosphate solutions; and since the total amount available was so very small and the percentages were computed on the basis of the available supply, any percentage which the plant might have absorbed would have been entirely inadequate to meet the nutritional demands of the plant for phosphorus.

The pH values for the leaves only of the corn of this study were obtained by using indicator dyes on free-hand sections. In the higher concentrations of phosphate the pH was near the average usually given for plants making a vigorous growth. The curve for the pH of the soil solutions fitted rather well that for the pH of the sap, if the measurement of the latter is to be accurately estimated from the region of the bundles in the leaf. A slight shifting of the pH values in a dilute solution series had a more pronounced effect than in Knop's solution because in the former the phosphorus content was so low that the buffer effects of this high valence mineral were almost nil.

MEYER (**26**) discovered long ago that certain plants form little or no starch in their leaves and that when starch is found in the leaves the amount of sugar present is relatively low. The relationship between sugar and starch for the corn plants of this study was investigated. In almost every group there was an opposite tendency in the percentage concentration of the two compounds. Comparing the graphs for sugar content and starch content with that for sap acidity, it was evident that sugar content was high when sap acidity was high, and starch was present in greater amounts when

TABLE VII

CHEMICAL ANALYSIS OF THREE VARIETIES
(PERCENTAGES EXPRESSED IN TERMS OF DRY WEIGHT)

SOLUTION	VARIETY	AV. LIVE WT. IN GM.		ASH		NITROGEN		REDUCING SUGARS		STARCH		CALCIUM		POTASSIUM		PHOSPHORUS	
		TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT	TOP	ROOT
		gm.	*gm.*	%	%	%	%	%	%	%	%	%	%	%	%	%	%
−P(a)	H	40.0	17.9	15.6	39.9	2.41	0.35	4.5	8.1	20.5	17.5	1.43	2.65	0.15	0.19	1.05	1.32
−P(b)	H	10.8	11.3	20.5	20.2	1.57	1.23	3.1	3.6	16.2	21.1	1.69	1.39	0.13	0.38	0.76	0.77
−P(d)	H	8.6	11.1	15.6	14.4	3.09	2.19	5.1	8.2	20.8	25.1	1.91	2.11	0.14	0.59	0.19	0.16
8 P	H	63.0	29.5	18.4	40.1	1.86	0.54	5.0	3.8	21.3	18.5	0.73	2.26	0.25	0.38	0.30	2.12
Knop	H	24.8	15.0	12.6	27.0	1.91	0.70	5.2	3.1	20.8	16.8	1.58	1.49	0.31	0.12	0.45	0.46
0.25 P	H	26.6	17.3	11.5	24.4	1.76	0.84	8.3	2.5	16.9	23.0	1.82	2.73	0.22	0.22	0.24	0.41
0.10 P	H	15.8	12.5	13.4	19.3	1.78	0.76	5.6	5.0	18.9	24.8	2.03	2.14	0.19	0.53	0.18	0.13
−P(a)	O	19.9	15.0	17.1	12.5	2.29	0.71	5.1	9.4	20.0	19.3	2.22	2.82	0.14	0.34	1.06	0.60
−P(d)	O	20.1	9.7	11.1	12.3	1.60	0.91	5.6	3.3	16.3	20.5	1.18	2.08	0.10	0.25	0.31	0.11
8 P	O	46.7	21.7	16.1	18.8	1.73	0.71	4.6	3.6	18.0	19.6	0.61	1.48	0.24	0.22	1.01	1.21
Knop	O	43.2	20.0	7.5	33.5	1.85	0.84	5.4	2.9	15.1	14.6	0.88	2.70	0.12	0.25	0.18	0.30
0.25 P	O	34.0	20.0	9.7	22.7	2.02	1.42	6.3	4.1	16.7	18.3	1.31	3.67	0.22	0.44	0.19	0.13
0.10 P	O	32.3	22.3	9.4	14.0	1.92	1.74	4.6	3.2	21.9	20.4	1.19	2.68	0.12	0.34	0.24	0.15
−P(a)	J	34.8	17.8	19.6	36.1	1.91	0.41	2.4	3.5	23.3	16.7	1.81	2.56	0.21	0.33	0.12	1.19
−P(b)	J	11.8	10.1	11.4	24.8	2.46	0.80	2.4	3.9	16.3	22.0	1.21	2.05	0.11	0.86	0.48	0.39
−P(d)	J	13.2	11.1	12.5	15.2	1.90	1.17	2.8	9.1	15.0	18.6	1.32	2.75	0.10	0.14	0.34	0.30
8 P	J	48.7	20.6	15.4	26.8	1.42	0.73	4.6	5.1	14.9	19.6	0.69	2.10	0.98	0.23	1.08	1.70
Knop	J	18.4	15.3	13.8	32.3	2.39	0.49	2.1	2.0	15.7	16.9	1.08	1.61	0.15	0.16	0.48	0.67
0.25 P	J	33.4	27.0	10.1	18.3	1.69	0.74	7.9	4.1	16.8	22.7	1.67	4.49	0.14	0.30	0.20	0.41
0.10 P	J	24.0	16.0	9.5	15.4	2.11	1.42	10.0	5.5	16.9	18.2	1.09	2.24	0.19	0.36	0.18	0.14

the pH was high. KRAYBILL (18) found, on analysis, that low-phosphorus plants were seen to be high in reducing sugars and sucrose, and low in starch. Low-phosphorus plants were shown above to have under normal conditions a high acidity. In the plants of the lower phosphate concentrations, starch content usually exceeded the sugar content, and in the unit phosphate group the sugar was present in larger amounts than the starch. This situation was reversed, however, in the unit phosphate H series.

It was discovered that all plants making their entire growth in minus phosphate solution contained some phosphorus. The chief source of this phosphorus supply was the seeds. Seeds of varieties H, N, P, and U were found to have a phosphorus content of from 0.40 to 0.57 per cent. of the dry weight (table VIII). H and U had the same average percentage, which was below the general average of 0.45 per cent.

TABLE VIII

ANALYSIS OF SEEDS

(ALL WEIGHTS EXPRESSED IN MILLIGRAMS)

VARIETY	STORAGE WT. OF SAMPLE	DRY WT. OF SAMPLE	WEIGHT OF ASH	PERCENTAGE OF PHOSPHORUS	WT. OF P PER SEED	WEIGHT OF P IN SAMPLE
	mg.	*mg.*	*mg.*	%	*mg.*	*mg.*
H	1471.8	1334.9	17.1	0.43	0.727	5.815
N	1769.4	1638.0	24.6	0.57	1.157	9.255
P	2340.7	2128.9	33.5	0.40	1.069	8.555
U	1671.4	1529.3	19.7	0.43	0.817	6.535

The percentage of phosphorus absorbed, of that available, was moderately high in the unit phosphate concentration series and always dropped low in the five-hundredths phosphate or minus phosphate groups. The tendency for the percentage of phosphate absorbed to rise in minus phosphate P and minus phosphate U was shown in a marked way by the luxuriant growth of these two groups (fig. 1). The average percentage of phosphorus absorbed in the five concentrations for each variety was as follows:

Variety	Av. percentage
H	13.6
N	22.3
P	25.6
U	32.5

Aside from all variations then in absorption rate caused by light, temperature, oxygen, carbon dioxide relations, and other factors, there are real varietal differences.[1] HOPKINS (**14**) made no allowance for these differences when he estimated that a corn crop producing a hundred bushels of corn and three tons of stover per acre would remove from the soil 148 pounds of nitrogen and 23 pounds of phosphorus.

A few other series in which plants made rather satisfactory growth were studied to determine the variation in the capacity of these under different nutrient conditions for removing phosphorus from the solutions (table IX).

TABLE IX

PHOSPHORUS ABSORBING CAPACITY OF VARIETIES
(ALL WEIGHTS EXPRESSED IN MILLIGRAMS)

VARIETIES		P FOUND IN SEEDS	P OFFERED IN SOLUTIONS	TOTAL WT. OF P OFFERED	WT. OF P FOUND IN PLANT	PERCENTAGE ABSORBED OF P OFFERED
		mg.	*mg.*	*mg.*	*mg.*	%
−P (a)	H	0.4362	262.80	65.8091	59.42	90.3
−P (b)	H	0.4362	98.59	24.7565	21.13	85.3
8 P	H	0.4362	657.25	164.4215	125.51	76.3
−P (a)	O	0.6896	350.50	87.7974	31.39	35.7
8 P	O	0.6896	888.70	222.3474	81.33	36.7
−P (a)	J	0.6942	197.17	49.4661	18.65	37.7
−P (b)	J	0.6942	65.73	16.6061	10.25	61.1
8 P	J	0.6942	525.80	131.6235	94.70	71.1

It was found that the percentages of phosphorus removed of that offered to the plants were 36.1 for variety O, 56.6 for variety J, and 84.0 for variety H. These are roughly in the ratio of 3 : 5 : 7. It was shown, on analysis of four strains above, that variety H was the least efficient of the four, but here it was seen to be more efficient than either variety O or variety J. From this comparison, N is seen to be more responsive to phosphorus feeding than J, its reciprocal cross.

When H was found to have removed only 13.6 per cent. of the phosphorus offered in the solutions when fed with 1 P, 0.25 P, 0.1 P, 0.05 P, and −P variations in phosphate concentrations, and to have removed 84 per cent. from the solutions when fed with the −P (a and b) and the 8 P series, a great discrepancy in one or the other procedure seemed certain. The differences in the time of offering, and the concentration of phosphates in the two types of nutrient solutions, are sufficient alone to explain these apparently

[1] STRINGFIELD and SALTER (Jour. Agr. Res. 49: 991–1000. 1934) have shown that differential varietal responses in yield of corn among four variety levels was significant in at least two seasons.

contradictory data, for although H has a high phosphorus demand, it would surely take up large quantities from a solution in which phosphate was eight times as concentrated as Knop's solution. A comparison of the roots formed in the former class of solutions with those formed on H in the latter class of solutions was alone ample evidence that there would be an enormous difference in the absorbing capacity of this strain under the two types of treatment.

It was also observed that phosphates were not absorbed in the same proportion as they were provided in the nutrient solutions (table X).

TABLE X

RELATIVE RATE OF ABSORPTION TO AVAILABILITY

	1 P	8 P	−P (a)	0.25 P	0.10 P	−P (b)	0.05 P	−P
Relative strength of P in solutions	394	24	9	9	8	3	2	0
Relative absorption of P	102	14	5	7	4	2	6	3

Using the average dry weight per plant, the T/R, the C/N, and the K/Ca ratios were computed for each series (table VI). High T/R ratios were generally found for the series having the higher total dry weight in grams, although there was not a close correlation between them. The lowest T/R ratios were found from plants whose roots generally contained a larger percentage of inorganic elements than the tops (table VII), a condition which MÜLLER (**27**) believes is an indication that the assimilation products were stored in the roots and not utilized for new leaf production. This increase, he contends, in the proportion of dry matter in root per unit leaf area is the principal immediate cause of reduced growth.

Considering the total dry weight of each plant as a measure of growth, the three 8 P solution growths (table VI) were among the best growths, with dry weights of 8.09, 7.7, and 7.81 gm. for H, O, and J respectively. The C/N ratios of these three in turn were 17.2, 6.9, and 16.2 with an average of 10.1. The − P series also made good growths with an average ratio of 14.8, and, likewise, Knop's solution plants with an average ratio of 13.1. The average of the averages was 12.6, which probably is very near the optimum C/N ratio for the plants of this experiment.

When it was obvious that variety H was not making a good growth in any one of the five phosphate concentrations (fig. 1) some seeds of this variety were planted in a pot and fed with Knop's solution prepared with double the usual amount of potassium acid phosphate, other salts remaining constant. This corn had reached an average height of 20 cm. in the same period of time required by unit phosphate P, the best growing variety

(fig. 1) to attain the same height. It is evident, therefore, that the concentration of phosphate was not high enough to insure maximum growth of variety H in Knop's solution (table I). A comparison of variety H plants with those of N, P, and U (fig. 1) reveal that even in Knop's solution variety H was a less vigorous plant than most of those plants of the other strains which grew in the lower phosphate concentrations. Strain H possesses very limited capacity for phosphorus absorption from dilute solutions because of its high phosphorus demands.

EYSTER (**6**) has found a genetic type of maize that digests and absorbs the endosperm, but is unable to utilize the sugars thus obtained. With ample evidence in this study to demonstrate that sugars accumulate more in P-deficient strains of corn than in P-efficient lines, it is reasonable to suppose that the cause of phosphorus deficiency in any inbred line or hybrid is an inherited lack of capacity for sugar metabolism. In ZIMMERLEY'S (**40**) experiments with truck crops, heavy applications of phosphate resulted in an increase in photosynthetic activity, for the plants given such treatment were found to contain a much larger content of starch and total sugars.

It has been suggested that a high ratio of secondary to primary roots may be the cause of phosphorus efficiency in inbred lines of corn, and, further, that inheritance of branched root type is a probable cause of efficiency in corn hybrids (**35**). In the various series of this study noticeable contrasts were manifested by the root systems of three varieties, the plants growing in the 8 P solutions being typical examples of these differences. Some main roots were selected at random from the root masses of H, O, and J varieties that had grown in 8 P solutions, and the secondary roots emanating from the fifth centimeter below the crown were counted with the following results:

	Varieties		
	H	J	O
Average length of root mass in centimeters	26	24	21
Average number secondary roots 4 cm. below the crown	11	9	8

There is a correlation, therefore, between phosphorus efficiency and the number of secondary roots. These data confirm the opinion of SMITH (**35**). More experimental evidence is needed, however, to fully establish this principle.

LIVINGSTON (**19**) states that, "No certain fertilizer can be recommended as the best corn fertilizer. Some fields require one element of plant food,

while others may require a different treatment. In other words, the soil should be fertilized and not the corn." This conception of LIVINGSTON'S is one that has long been held by many agronomists. Recent extensive literature (**1, 7, 12, 17, 30, 31**) has emphasized other phases of the problem of plant nutrition such as quantity, method of application, time of application, general reactions of nutritive elements and their effect upon each other, the reaction of the soil solution and its effect upon the hydrion concentration of the sap, evidences of distress in the growing plant—symptoms of deficiency, toxicity, and the like. All of this, to a greater or less degree, has revolutionized and rationalized fertilizer practices and put them on a more effective basis, centering attention more upon the plant and its requirements than upon the soil and its relative state of fertility. This procedure is not only more scientific but more logical because a plant possesses considerable ability to adjust itself to culture media of different concentrations.

LOEHWING (**20**) in a rather extensive study of calcium, potassium, and iron balance on growth and composition of wheat, corn, and clover on four types of muck soil concluded that a study of the chemical composition of the plant was more valuable than soil analysis. LIVINGSTON (**19**) merely advocated the application of fertilizers with some emphasis upon what to use, but no reference as to how, when, or why they should be utilized.

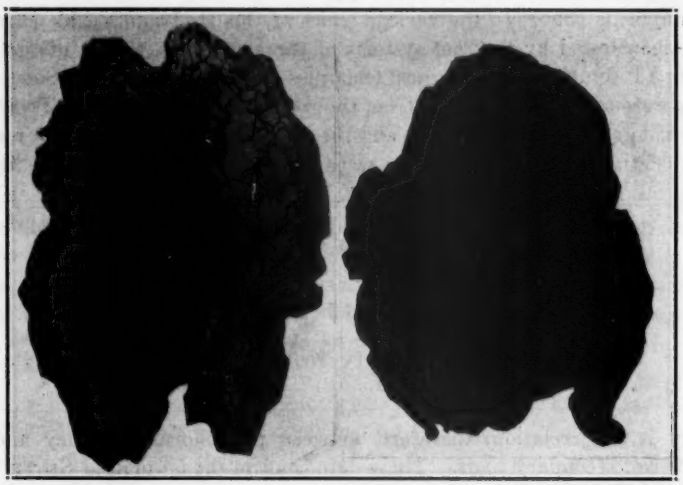

FIG. 2. Transverse sections of roots of variety U: left, section of quarter phosphate series showing lighter cell walls of more nearly normal roots; right, section of minus phosphate series showing heavy cell walls which usually are found in phosphorus-starved plants (**5, 37**). Both × 50.

Careful experimentation will reveal the fertilizer requirements of every variety to be grown, and, if this is known in advance by the grower, much time and money will be conserved and the same or vastly superior results will be attained. P-deficient varieties will probably never do well on soils of low fertility but, on the other hand, phosphorus-efficient strains may not make as high yields on soils with a high natural fertility as those less responsive to phosphorus feeding.[2] Different forms of phosphate may be made available for corn in the field by many different methods but there is always some best procedure for every variety and the present study has shown that even plants of the same variety respond to the same treatment in distinctly different fashion.

Summary and conclusions

1. Twenty-one varieties of Reid's Yellow Dent corn were grown in quartz sand cultures. Knop's solution, with varying concentrations of phosphate, was supplied as needed and varieties showing evidence of phosphorus deficiency were analyzed. Later certain varieties, which had been isolated because of characteristic behavior, were grown in different concentrations of potassium, phosphorus, nitrogen, and calcium, part of the variation in treatment being the application of an adequate supply of each of these elements at different periods in the growth cycle of the plants.

2. Three varieties selected for special study, when compared with each other, showed the capacity for absorbing phosphates from sand cultures of similar concentration in about the ratio of 3 : 5 : 7.

3. The rate of phosphorus absorption was not directly proportional to the phosphate concentration of the culture solutions.

4. Heavy concentrations of phosphorus constantly available from the beginning of the growth cycle produced a rapid growth at first, but later a reduced growth rate and other symptoms suggested phosphorus toxicity.

5. A high correlation was found between the phosphorus content and the growth of corn expressed in terms of its dry weight.

6. Plants with a high sugar content were at the same time relatively low in starch and vice versa.

7. A high correlation was found between the phosphorus-absorbing capacity of each series and the pH of the soil solution.

8. Excessive transpiration caused by continuous and heavy applications of nitrogen produced early death in corn.

9. Responses exhibited as accelerations of growth rate were more pronounced when heavy applications of potassium and phosphate were made after brief starvation periods than when either nitrogen or calcium was so applied.

[2] STRINGFIELD and SALTER (*loc. cit.*) have gathered experimental data to prove that a variety may be relatively better at low than at high fertility levels.

10. Dilution and acidity of the soil solution were both found to play a prominent rôle in the regulation of the rate of absorption, both operating to decrease it and likewise to diminish the growth rate of the plants.

11. Photomicrographs of stem, leaf, and root sections showed much starch, well-developed xylem, and light to medium cell walls in plants growing in the higher phosphate concentrations, and little starch, poorly developed xylem, and medium to heavy cell walls in plants growing in the more dilute solutions.

12. When two inbred types were crossed by plant breeders, if they used one markedly P-deficient strain and another as definitely P-responsive, the gene for P-deficiency behaved as a recessive in the F_1 generation hybrid, demonstrating the beneficial effects in this instance of hybridization. This problem needs further study.

13. There was a close correlation between phosphorus response and the number and character of secondary roots, probably a hereditary characteristic, which suggested a reason for the varietal differences in phosphorus-feeding capacity.

14. Requirement of a variety of corn for the phosphorus necessary to accelerate respiration to the point that sugars present might be utilized in the metabolism of the plant probably is a cause for differentiation of varieties in responsiveness to phosphate fertilization.

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UNFROZEN WATER IN APPLE SHOOTS AS RELATED TO THEIR WINTER HARDINESS[1]

ARVIL L. STARK

(WITH NINE FIGURES)

Introduction

In breeding apples for a region where winter hardiness is an important consideration, the desirability of having some rapid and reliable means of separating the winter hardy seedlings from more tender specimens is obvious. Any elimination that can be made in the first year or two of seedling growth is economical from the standpoint of both time and cost.

A number of different methods and procedures have been employed in an attempt to separate cold resistant plants from those less resistant to freezing temperatures. A survey of the literature related to this problem suggests that the capacity of a plant to retain its moisture against the extracting forces of freezing is associated with its ability to survive cold. With this generalization in mind the present investigation was undertaken to ascertain whether or not winter hardiness in apple shoots could be related to the quantity of water remaining unfrozen at low temperatures.

The meaning of the term "bound water" used in the literature is controversial and will, therefore, be avoided in this discussion. "Water-retaining capacity" as used subsequently refers to the capacity of a tissue to retain water in the unfrozen state when subjected to certain freezing temperatures.

Literature review

Good bibliographies and reviews of the general literature on the influence of freezing temperatures on plants may be found in the publications of CHANDLER (**4**), HARVEY (**16**), MAXIMOV (**29**), NEWTON (**34**) and ROSA (**43**). An excellent discussion of the literature more closely related to the problem of winter hardiness in apple shoots is given by HILDRETH (**18**). JONES and GORTNER (**24**) included in their paper a comprehensive summary of the work published on the effect of freezing on colloidal systems. GORTNER (**12**) considered also the water relationships in colloidal and living systems. Recently he (**11**) explained the nature and the methods of estimating bound water. Critical examination of the methods of measuring and the meaning of the term "bound water" are discussed by BRIGGS (**2**), GROLLMAN (**13**), NEWTON and GORTNER (**37**), and HILL (**19, 20**).

The literature reviewed here is of more particular interest to the study presented in the following pages. ROBINSON (**42**) showed that winter hardi-

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ness of insects was related to their ability to retain water in the unfrozen state when subjected to freezing temperatures. By the heat-of-fusion method he found that tender species were unable to increase their capacity to retain water in the unfrozen state when subjected to a temperature of $-20°$ C. In contrast to these, hardy species exhibited considerable increase in this capacity when hardened under the same conditions as the tender species. Using the dilatometric technique, SACHAROV (**45**) obtained results in agreement with ROBINSON, although different species of insects were studied.

From his investigations on the winter hardiness of wheat plants, NEWTON (**34**) concluded that the volume of press-juice obtainable from hardened leaves was inversely proportional to the hardiness of a variety. The quantity of hydrophilic colloids contained in the press-juice, as measured by the method of NEWTON and GORTNER (**37**), was found to be directly proportional to hardiness. By means of the dilatometer, NOVIKOV (**39**) showed that the winter wheats which were more resistant to cold contained the greater quantities of unfrozen water when subjected to a temperature of $-5.75°$ C. The amount of unfrozen water increased with the duration of hardening in the resistant group but very little or no increase was found in the non-resistant varieties.

LOTT (**26**), working with brambles during the dormant season, observed a direct correlation between hardiness and percentage of unfrozen water as measured with the dilatometer at $-6°$ C. WEIMER (**59**) concluded from his studies on alfalfa roots that unfrozen water at $-5°$ C. was related to the degree of hardening, but it could not be used to differentiate between hardy and non-hardy varieties. An increase in the quantity of unfreezable water during the hardening process in cabbage was brought out by ROSA (**43**), who determined the frozen water at $-3°$, $-4°$, $-5°$ and $-6°$ C. with the dilatometer. He concluded that the rates of decrease in percentage of freezable water coincided with the observed rate of hardening.

Materials and methods

The material used throughout this investigation consisted of shoots from 15 standard horticultural varieties of apple, *Malus sylvestris*. These varieties were selected so that degrees of hardiness from very tender to extremely hardy would be included. In descending order of their hardiness, as based upon years of field observation at this station (**27**), the varieties may be arranged as follows: Hibernal, Virginia, Shield's Crab, Dudley, Okabena, Wealthy, Ioensis, Wolf River, Cortland, Baltimore, Jonathan, Delicious, Grimes, Wagner, and Stayman.

Material subsequently referred to as "nursery shoots" consisted of shoots taken from stocks planted in 1924 and cut back to the crown each winter. Shoots of all varieties from this source averaged about 3 feet in length at

the end of the growing season. The term "tree shoots" applies to tip growth of a single season from trees planted in 1926. These shoots were in general about 18 inches in length at the close of the growing season. Only 10 of the 15 varieties were available in tree form. Those not available were Hibernal, Shield's Crab, Okabena, and Ioensis. The tree and nursery material was grown in adjacent plots and unformity of soil and other conditions was considered in collecting samples.

Shoots were collected before 9:00 A.M. on the day of determination and the leaves were immediately removed. After the shoots of a variety were weighed roughly to 60 gm. they were cut into three samples of approximately 20 gm. each. Each sample was then quickly weighed on a torsion balance and transferred immediately to a stoppered test tube. As soon as the three samples of one variety were weighed, two of the tubes were placed in the cooling bath at $-20°$ C. and the third in a refrigerator until used in a later treatment. Each sample weighed between 20 and 20.3 gm. and consisted of pieces about 1 inch in length.

The entire growth of the current year was used in sampling, except where a smaller portion was needed to bring the sample to the desired weight. In this instance a midsection of another shoot supplied the deficiency. The necessity of this procedure in sampling was indicated by a series of preliminary determinations in which it was shown that the water relationships of tip and basal portions of the same shoot were different. The mean difference in unfrozen water between all duplicates was found to be 0.80 per cent., with one-half of the observations falling between 0.4 and 1.2 per cent.

The heat-of-fusion method was used throughout this investigation to determine the values for frozen water. The first recorded attempt to apply this method to biological tissue is attributed to MÜLLER-THURGAU (**33**). Later modifications and improvements have been made by RUBNER (**44**), THOENES (**56**), and ROBINSON (**42**). HILL (**20**), ST. JOHN (**47**), and MEYER (**31**) have suggested improvements and criticisms. A detailed description of the procedure followed in this study has been presented in two previous papers (**51, 52**).

In brief, the heat-of-fusion procedure consists of measuring the change in temperature of a known quantity of water caused by the addition of the frozen material. With the above information plus the temperature, weight, and specific heat of the material it is possible to calculate the quantity of frozen water in the sample. The frozen water value is subtracted from the total water content of the sample to ascertain the value for unfrozen water.

Experimentation

EFFECT OF SLOW AND RAPID FREEZING ON UNFROZEN WATER

It has been shown (**3, 4, 18**) that the rate of freezing is a factor in determining the degree of injury in plant tissue. Non-living gel systems are

also known to be affected differently by slow and fast rates of freezing (**14, 32, 54**).

A number of trials with apple shoots in the dormant condition failed to reveal any significant difference between duplicates, one set of which was cooled from 0° C. to – 20° C. in 12 hours while the other was frozen immediately at – 20° C. Apparently the rapid rates of cooling employed here had no measurable influence on the quantity of ice found in the tissue. In this connection, however, it should be pointed out that slow temperature drop is not necessarily slow freezing because of the possibility of under-cooling. JOHNSTON (**23**) observed incipient ice formation in peach buds at –5.9° to – 8.0° C. From preliminary studies with the dilatometer it is known that solidification began in these shoots between – 4° and – 6° C.

EFFECT OF PERIOD OF FREEZING ON UNFROZEN WATER

There is some evidence (**18, 36, 40**) that prolonged freezing periods bring about injury to plants. HILDRETH (**18**) observed greater injury in apple shoots when in the hardened condition after a freezing period of 12 hours at – 43° C. than after a shorter period of 3 hours. If the increased injury is caused by greater water loss from the protoplasm through ice formation, it may be expected that more ice will form with longer exposures to the freezing temperature.

During the growing season (May 23) a water-ice equilibrium was reached in the shoots by a freezing period of 4 hours at – 20° C. In the dormant condition (Jan. 18), however, a water-ice equilibrium was not attained in a 4-hour period. In the January trials 2.8 per cent. more water was removed in 24 hours of freezing than during a corresponding 4-hour period. A statistical analysis of the data obtained proved this difference to be highly significant. The value for t was 9.77 while the highly significant value for t in this instance was only 2.97 (**10**).[2] An additional 72-hour period of freezing after the first 24 hours failed to freeze more water from the tissue.

Evidently the time required for the attainment of a water-ice equilibrium at – 20° C. varied with the condition of the tissue. When the shoots were in the hardened state more time was necessary for the establishment of this equilibrium than when it was more readily killed by cold. Perhaps the longer freezing time required to reach equilibrium in the shoots while in the hardened condition is a factor in their surviving cold. The influence of time is important in a study of heat treatments on colloidal and living systems and it is possible that death from cold is a time-temperature relationship as is death from heat.

[2] t is a value used in the test for significance of differences between means. See FISHER (**10**).

The percentage of moisture in the tissue is also probably a factor in determining the time for attainment of equilibrium. JONES and GORTNER (**24**) found that the time to reach a water-ice equilibrium in gels varied with the concentration and the temperature of exposure. At temperatures near the freezing point the rate of reaching equilibrium was slower than at colder temperatures. Gels of higher concentration froze more slowly than those with less dispersed material.

In this respect the behavior of apple shoots resembles that of gels in that it requires more time to reach a water-ice equilibrium when the moisture content is lower. On the other hand, observations in apple shoots frozen at – 5° C. proved that equilibrium at this temperature was more rapid than at the colder temperature of – 20° C. This is just the reverse of the behavior with gels as observed by JONES and GORTNER (**24**).

INFLUENCE OF COLD TEMPERATURES ON WATER-RETAINING SUBSTANCES

To measure the behavior of apple shoots when subjected to a previous cold temperature and then frozen at a milder temperature, a sample was cut in triplicate. Two of the three were frozen at – 20° C. for 4 hours and then the freezing bath was allowed to warm to – 5° C. When the latter temperature was reached one set of samples was taken from the bath and placed in a water bath at room temperature for one hour. At the end of the hour this set of tubes was returned to the – 5° C. bath along with a triplicate not yet frozen. All three sets were then frozen 14 hours at – 5° C.

The close similarity of the triplicate samples in table I is evidenced by a comparison of the percentages of water within a variety.

TABLE I

INFLUENCE OF PRECEDING LOW TEMPERATURES ON UNFROZEN WATER. MAY 3, 1933

VARIETY	TREATMENT					
	I		II		III	
	– 5° C. ONLY		– 20° C. THAWED		– 20° C. NOT THAWED	
	WATER	UNFR. WATER	WATER	UNFR. WATER	WATER	UNFR. WATER
	%	%	%	%	%	%
Hibernal	53.4	47.6	53.1	43.1	53.0	37.7
Virginia	55.0	44.0	55.3	39.6	55.3	30.8
Dudley	53.0	46.5	52.6	42.3	52.4	35.2
Wealthy	52.0	49.0	51.5	43.6	51.2	38.2
Ioensis	51.9	49.2	51.6	41.9	52.2	36.2
Delicious	53.3	46.5	52.7	43.0	53.2	33.5
Stayman	51.3	45.3	51.0	38.6	51.4	38.9
Mean	52.8	46.9	52.6	41.7	52.7	35.8

A comparison of the values for unfrozen water in treatments I and II indicates that the prefreezing at – 20° C. caused some alteration in the tissue that impaired its water-retaining capacity against subsequent freezing at – 5° C. This alteration is shown by the fact that the set frozen at – 5° C. only was able to hold 46.9 per cent. of the water unfrozen, while the set previously cooled at – 20° C. could hold unfrozen only 41.7 per cent. of the water. Thus it is seen that the process of freezing out and reabsorption of water by the tissue is not reversible.

Evidence of a partial reversibility, however, is obtained from the fact that part of the water originally frozen at – 20° C. was reabsorbed and not frozen out again by the – 5° C. temperature. This is clear from a comparison of the unfrozen water values in treatments I, II, and III. Here it is seen that the mean value for unfrozen water in the – 20° C. thawed set (41.7) is approximately midway between the means of the – 20° C. not thawed set (35.8) and the – 5° C. only set (46.9). If all of the water originally frozen at – 20° C. in the thawed samples had been formed as ice again in the refreezing at – 5° C. it might be expected that the two means for unfrozen water in treatments II and III would be the same. Since approximately one-half of it remained unfrozen at – 5° C., a partial reversibility is evident.

JONES and GORTNER (**24**) were unable to demonstrate complete reversibility in the gelatin systems they studied. In systems of inorganic hydrogels they found the quantity of water frozen to be increased by a previous lower temperature. The behavior of apple shoots is similar to that found in inorganic hydrogels as reported by JONES and GORTNER (**24**).

The data in table I are also of interest from the view-point of winter injury. If cold injury results from a dehydration of the protoplasm by the removal of water in the formation of ice, a period of severe cold followed by warmer weather that remained below freezing would probably be as injurious as if the lower temperatures had continued. The basis for this conclusion is apparent from treatments I and III, where it is shown 11.1 per cent. less water remained unfrozen in the set previously frozen at – 20° C. than in the – 5° C. only set. The value for unfrozen water when measured at –20° C. on this date was approximately the same as the unthawed treatment at – 5° C. in table I.

Thus it would seem that once the water has been frozen it remains in the solid state at a warmer temperature than could have frozen it originally. This failure of the ice to melt indicates that colligative properties are of minor importance in retaining the water in the liquid state. If they were effective, melting of the ice could be expected at the warmer temperature. Results similar to those contained in table I were obtained with a temperature interval of – 20° and – 43° C.

Unfrozen water at −43° C.

A freezing temperature of −20° C. was used for the most of the determinations in this study, principally because Rubner (**44**), Thoenes (**56**), and Robinson (**42**) have assumed that all of the free water and none of the bound water is frozen at this point. Gortner (**12**) has stated, ". . . when a part of the bound water is removed from the hydrophilic colloid, the colloidal structure is altered and vital function interfered with."

A number of observations were made during January, 1933, at −43° C. to ascertain whether or not additional water could be frozen out of the tissue over that removed by −20° C. The temperature lowered from −20° to −43° C. brought about an average decrease of 12.5 per cent. in unfrozen water. If this additional quantity frozen out by the 23° C. drop in temperature is added to 22.5 per cent., the mean difference between −5° and −20° C. at this time, the total decrease in unfrozen water from −5° to −43° C. is 35 per cent. In other words the unfrozen or bound water decreased very decidedly with temperature lowering.

Jones and Gortner (**24**) found the quantity of unfrozen water in gelatin gel to change very little, if at all, between −6° and −50° C., while in silica gel and other gels of the inelastic type unfrozen water decreased with temperature lowering. Again there is evidence that the behavior of the substances responsible for retaining water unfrozen in apple shoots is similar to the inelastic type of gel rather than to the elastic type.

From the view-point of winter hardiness −43° C. may be a more satisfactory temperature than −20° C. for comparison of varieties during January. Hildreth (**18**) showed that shoots from a tender apple variety were killed by a temperature of −41° C. in January, while a hardy variety survived this temperature. The data collected at −43° C., however, did not allow the varieties used in this study to be properly arranged in order of their hardiness.

Influence of a preceding warm temperature on unfrozen water

It is commonly observed that injury to plants from cold will result if a warm spell during winter is followed by low temperatures which would not have been injurious in the absence of the mild period. Injury of this nature is especially prevalent on peaches and plums in a region where the temperature fluctuates over a wide range during the winter. Under such conditions some alteration in the water relationships should occur if the injury is the result of water loss in the formation of ice.

To measure the influence of a warm period, as it affects the water-retaining capacity of the shoots when frozen, four shoots of each of seven varieties were collected on January 13, 1933. In two of these sets unfrozen water was measured immediately. One set was placed with the cut ends in water

in cold storage at 0° C., the other set remained in the laboratory at room temperature. After a storage period of 11 days unfrozen water in these last two sets was measured at – 20° C., as in the first two.

TABLE II

EFFECT OF A PREVIOUS WARM SPELL ON WATER-RETAINING CAPACITY AGAINST FREEZING AT —20° C.

VARIETY	JAN. 13, 1933				JAN. 24, 1933			
	SAMPLE I		SAMPLE II		ROOM TEMPERATURE		0° C.	
	WATER	UNFR. WATER	WATER	UNFR. WATER	WATER	UNFR. WATER	WATER	UNFR. WATER
	%	%	%	%	%	%	%	%
Hibernal	47.5	43.6	46.3	41.5	53.3	37.9	51.4	37.7
Virginia	51.6	36.0	51.0	37.5	57.0	27.1	53.9	34.8
Dudley	50.2	39.4	45.8	45.5	56.1	29.7	51.5	38.9
Wealthy	47.4	41.7	47.4	45.2	54.8	29.3	50.8	40.2
Ioensis	48.8	41.2	47.4	41.9	53.6	32.1	52.5	38.7
Delicious	45.7	42.6	50.2	35.5	56.4	30.5	52.6	36.3
Stayman	46.7	39.8	47.1	42.0	54.0	30.4	52.2	36.4
Mean	48.3	40.6	47.9	41.3	55.0	31.0	52.1	37.6

Comparison of the data given in table II in terms of t is as follows:

COMPARISONS	VALUES OF t
Sample I and sample II	0.50
Room and sample II	4.75
0° and sample II	0.53
Significant	2.14
Highly significant	2.98

In the data presented in table II the accuracy of replication in sampling is observable from a comparison of samples I and II. No difference of statistical significance was found between these two sets.[3] Similarly the samples stored at 0° C. were not significantly different from set II in grams of water held unfrozen. The samples held at room temperature, however, showed a decided decrease in water-retaining capacity when frozen at – 20° C. The highly significant value for t of 4.75 is evidence of this fact. It is probable that a warm spell in winter brings about a similar change in

[3] Tests of significance of differences in these comparisons were based on grams of unfrozen water rather than percentages because of the increase in total water in the stored samples.

the water relations of shoots, and, if true, the association of water-retaining capacity with ability to survive cold becomes apparent.

Unfrozen water as a means of separating hardy and tender varieties

To obtain a knowledge of the changing water relations throughout the year, determinations were made on all varieties at monthly intervals. By this procedure the rate and time of maturity, as indicated by water relationships, would be disclosed together with the relative water-retaining capacities of the different varieties.

In figures 1, 2, and 3 may be seen the course of the total water and the unfrozen water held at $-20°$ C. throughout the year. The three varieties selected for figures 1, 2, and 3 represent a very hardy variety (Virginia), a very tender variety (Stayman), and one intermediate in its ability to survive cold (Wolf River), as observed from years of horticultural field experiences at the Iowa Agricultural Experiment station (**27**).

Although there is a difference in the course of the curves of the three varieties, no single variety maintains its relative position consistently throughout the year. The interlacing of these curves is characteristic of all the fifteen varieties tested. The relative position of the varieties changed from month to month and at no time could a hardy variety be distinguished from a tender one by the percentage of unfrozen water in each. Not only was this true when unfrozen water was expressed as a percentage of the total water, but also when expressed as a percentage of the dry weight, as a percentage of fresh weight, or in grams of water.

Statistical analysis of varietal data

Even though it is impossible to separate varieties into an order of hardiness, a statistical analysis of variance indicates that varieties differ significantly in their capacities to retain water against freezing. Results of the analysis are shown for the three shoots as well as for the nursery shoots for both years (table III).

In the test for significance of varietal differences it is necessary to compare the value (1429) listed under "mean square" with that of "interaction" (203) in the same column. In like manner the significance of the difference between months may be tested. By these tests there was found in all three series a highly significant difference between varieties. Tests for significance were made in accordance with Fisher's method, but using the tables for "F" given by Snedecor (**50**). This is statistical evidence that varieties differ significantly with respect to their water-retaining capacity against freezing, although it is not possible to arrange them in an order of hardiness on this basis. In spite of the fact that this analysis does not explain at what time or where the differences occur, it does prevent

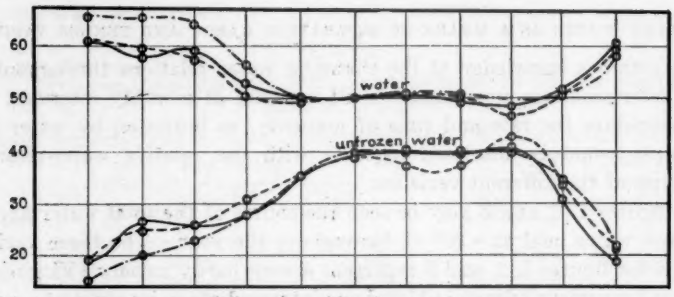

FIG. 1. Percentage of total and unfrozen water, 1931–32.

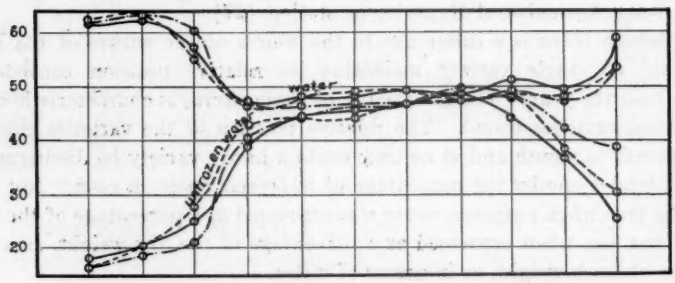

FIG. 2. Percentage of total and unfrozen water, 1932–33.

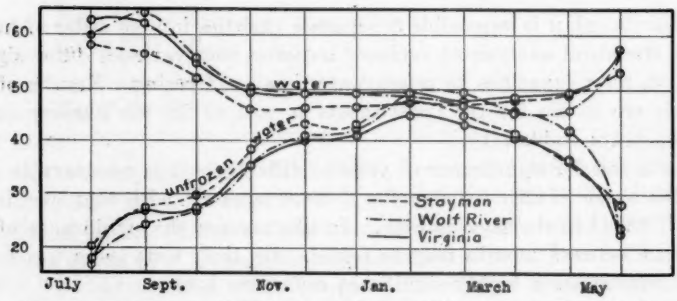

FIG. 3. Percentage of total and unfrozen water in tree shoots, 1932–33.

TABLE III

ANALYSIS FOR VARIANCE IN VARIETIES TESTED

VARIANCE	NURSERY SHOOTS				TREE SHOOTS	
	1931–32		1932–33		1932–33	
	D.F.	MEAN SQUARE	D.F.	MEAN SQUARE	D.F.	MEAN SQUARE
Total	329	1412	359	2612	219	2375
Within pairs	165	70	180	107	110	139
Between means of varieties	14	1429	14	920	9	1811
Between means, months	10	40463	11	76663	10	44977
Interaction or experimental error	140	203	154	404	90	431

the mistake of concluding that varieties are all alike in this respect. The difference in unfrozen water between months is more obvious than that between varieties and is also proven highly significant by the same test. This latter significance would be expected from an inspection of the curves.

Errors of technique and sampling are shown in table III. Values listed after "within pairs" are indicative of the error resulting from laboratory technique alone, while those under "interaction" represent the biological error plus the error of laboratory technique. This latter combination is the best estimate of the "experimental error." SNEDECOR (**50**) has discussed in some detail the calculation and the significance of these two errors.

Perhaps these results of varietal comparisons should not be surprising, in view of the fact that DUNN (**8**) and HILDRETH (**18**) found the survival order among varieties to change from time to time when apple shoots were frozen and allowed to recover. Even so, NICHOLS and LANTZ (**38**) and DORSEY (**6**) have observed in apple trees that the degree of browning after winter injury is quite consistent for a variety. It should be pointed out, however, that the degree of browning is not necessarily a measure of survival since many shoots may be severely browned by the cold and yet recover apparently uninjured.

A difference will be noted between water relationships in the same three varieties when taken from the trees and from the nursery row (figs. 2, 3). This difference is probably brought about by environment and dissimilar cultural practices, and suggests that environmental as well as inherent characteristics are important in determining water relationships in the shoots.

Another basis for comparison of varieties was suggested by the work of ROBINSON (**42**) who found that it was the increase in capacity to retain

water against freezing, after a hardening treatment, that distinguished a hardy from a tender species of insect. A comparison of the increase in unfrozen water values from July to January failed to reveal any differences that could be used to separate resistant from non-resistant apple varieties.

Comparison of varieties at −5 to −20° C.

It has already been shown that the formation of ice in apple shoots increased with temperature lowering. Such a relationship indicates that part of the water freezes readily, while other portions form as ice only at lower temperatures. Further cooling, after the first part is frozen, would freeze that water which is retained more tenaciously and is removed only by the colder temperature. A test of the capacity of a shoot to retain water between a high and a low freezing temperature should give some measure of its ability to survive cold, since it is this last portion, held more firmly, that decides survival or death through freezing.

In determining the quantity of water removed between a mild and a more severe temperature, unfrozen water measurements were made in duplicate samples held at −5° and −20° C.

TABLE IV

Mean differences in unfrozen water between −5 and −20° C.

Variety	Order of hardiness	Mean difference in percentage of water unfrozen	Order of differences
Hibernal	1	12.0	8
Virginia	2	10.7	3
Shield's	3	9.9	1
Dudley	4	11.1	5
Okabena	5	10.9	4
Wealthy	6	11.6	6
Ioensis	7	9.9	2
Wolf River	8	11.8	7
Cortland	9	13.4	11
Baltimore	10	13.8	12
Jonathan	11	14.9	14
Delicious	12	13.0	10
Grimes	13	13.9	13
Wagner	14	12.1	9
Stayman	15	14.9	15

The mean difference column in table IV was derived from an average of four monthly determinations in 1932–33. Data of December, January, February, and March were used in this comparison. These months were selected because they cover the period of greatest resistance to cold.

It is obvious from the data that the varieties cannot be arranged in the proper order of hardiness. If a line is drawn under the value for Wolf River, however, it may be seen that varieties falling below the line have larger mean differences than those above. With the exception of Hibernal and Wagner the varieties fall definitely into two distinct groups. Comparing the two groups statistically gave a value for t of 5.41 with 3.01 as the highly significant value. This is statistical evidence that during the dormant season a tender variety can be distinguished from a more resistant one by the larger quantity of ice formed in its shoots in the 15° interval between $-5°$ and $-20°$ C.

The arbitrary position of the line of separation should not be overlooked in drawing conclusions from these data.

Course of water relationships throughout the year

For a general discussion of the course of the water relationships throughout the year the means of the 15 varieties are plotted in figures 4 and 7. Charts for the temperature and rainfall over the same period accompany these curves. Rainfall is illustrated as total precipitation per week, while the points on the temperature curves are determined as means of 10-day intervals for maximum and minimum temperatures as recorded by the United States Weather Bureau at Ames, Iowa.

The position of each point in figures 4 and 7 is determined by the mean of 15 varieties. With this number of observations a small difference in location of the point is likely to be of more significance than if the position were established by a single pair of observations of one variety.

These curves represent data from shoots of a single growing season. All new growth present in the spring was removed before the samples were cut. In this way the changes observed were not attributable to the presence of new succulent tissue but rather to alteration of the material of the same source as that studied through the preceding summer and winter. The unfrozen water was measured at $-20°$ C.

On most varieties buds were just beginning to enlarge on April 21 in 1933, and leaves were only partly developed on April 21, 1932. As observed from material in the field there is apparently no relationship between the hardiness of a variety and the time its buds open in the spring. Nevertheless the stage of development in the buds is reflected by the water relations in the shoots. The data obtained on April 21, 1933, showed that varieties having buds ready to open retained a smaller percentage of water unfrozen than those varieties exhibiting a less advanced stage of bud development.

This alteration in water-retaining capacity with spring growth is probably best shown by the abrupt drop in unfrozen water from March to April (fig. 7). This sudden change in water-retaining capacity against freezing

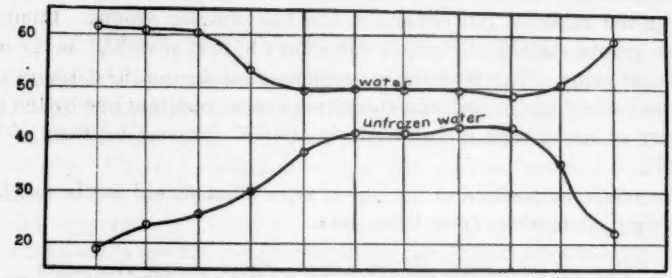

FIG. 4. Percentage of total and unfrozen water in nursery shoots, 1931–32.

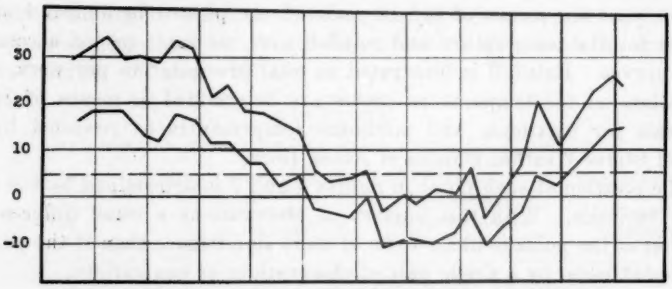

FIG. 5. Maximum and minimum temperatures, °C., 1931–32.

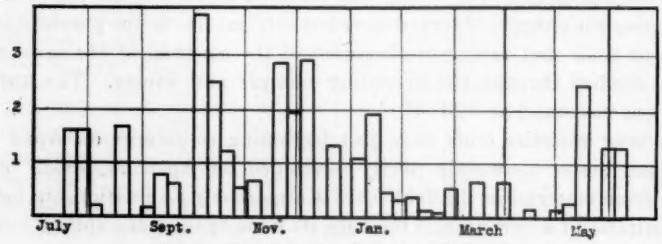

FIG. 6. Precipitation in inches, 1931–32.

takes place in spite of a slight decrease in the total quantity of water present. A similar decrease in percentage of water unfrozen is also noticeable in the spring of the previous year (fig. 4).

Another change in the water-retaining capacity of the tissue is noticeable in the autumn and early winter of each year. In early winter this change also takes place quite independently of the total water content of the shoots. Undoubtedly the cessation of food synthesis took place long before November, when many of the leaves had fallen and the remainder were dead from frost. Still, in both years there was a gradual increase in capacity to retain moisture unfrozen until March. This increase proceeded even after the water content in the tissue had reached the constant level maintained throughout the winter.

With the exception of the two examples just discussed the quantity of water remaining unfrozen appears to be rather closely related to the total water content of the tissue. This is shown by the fact that a low percentage of unfrozen water usually accompanies a high moisture content, while a high water-retaining capacity characterizes shoots having a low percentage of water. If the unfrozen water were merely a function of the total quantity present, it would remain unchanged after the water content of the shoots reached a constant level. In general this is the case, but the two exceptions noted in the spring and autumn furnish evidence that there must be some other influence on water-retaining capacity besides the percentage hydration of the tissue. In the autumn the exception is an increase while in the spring it is a decrease in capacity. These exceptions indicate that at least part of the substances retaining water unfrozen are associated with the vital or chemical activities of the tissue. If such were not the case it would be difficult to account for these alterations in capacity without an accompanying change in quantity of matter present. It is concluded, therefore, that living processes have an influence upon the substance responsible for retaining water unfrozen. This conclusion is contrary to observations of MEYER (**31**) on the leaf tissue of pine.

Of course it is impossible from the data on hand to account for the reactions responsible for this alteration in water-retaining capacity. It is suggested, however, that the protective action of sugar may be of some importance in this respect. Especially, in the light of a recent publication by NEWTON and BROWN (**36**), does the influence of sugar seem possible. They found in freezing expressed plant juices that less precipitation of proteins occurred after sugar had been added than when it was omitted. If such protective action existed in apple shoots, one might expect a maximum in water-retaining capacity during the time when sugar is known to be present in largest quantities. In an analysis of apple shoots HILDRETH (**18**) discovered that the highest sugar content occurred during the winter months. At

this time the water-retaining capacity against freezing is at a maximum, as seen in figures 4 and 7. The occurrence of the two maxima at the same time may be a mere coincidence, but there is a possibility of an interrelationship.

Along with the protective action of sugar it is possible that alterations in the materials present may cause some change in water-retaining capacity. In autumn, synthesis of substances into more complex structures could alter the physical and chemical state of the matter present without appreciably changing the dry weight. In the spring these same substances could be changed in the reverse direction by an analysis or breaking down process. This change in the state of the matter on hand could account for the alteration in water-retaining capacity without a measurable increase or decrease in percentage of dry matter.

Some unpublished observations of MARTIN (**28**) are of interest here. In a study of hardiness of sweet clover over a number of years he has found repeatedly, through cytological technique, that the protoplasm in the cells of the roots appeared very dense at the approach of winter. With the inception of growth in the spring he observed an abrupt liquefaction of the protoplasm. The exact time at which these changes took place varied with the weather conditions in late autumn and early spring. LOOMIS (**25**) has suggested that hardiness is the result of a structural differentiation of the protoplast which makes it more resistant to precipitation, such differentiation being dependent upon, and in part initiated by, a high sugar concentration in the tissue. These observations appear to be in agreement with the results of this study, but it should be mentioned, in opposition to this view, that HARVEY (**16**) believes the process of hardening is accompanied by an analysis rather than a synthesis of substances. He found more decomposition products of proteins in cabbage after hardening than in the unhardened leaves. NEWTON and BROWN (**36**), however, were inclined to attribute the presence of such intermediate products to the action of freezing and not to the hardening treatment.

No matter what the explanation underlying the changes in water-retaining capacity may be, it seems clear that it is associated with, or at least parallels, the hardiness of the shoots. HILDRETH (**18**) has plotted killing temperatures for apple shoots over the course of a year. His data show a killing temperature of $-3°$ C. in July, with a gradual lowering to about $-40°$ C. on the first of January. Little change is then noted until an abrupt upward trend begins the first part of April, reaching $-6°$ C. in May. The striking relation between HILDRETH'S killing temperature curve and the curves for unfrozen water in this study suggests that the ability to retain water against freezing may be of importance in cold-survival of apple shoots.

The greater resistance during the winter months is associated with a larger water-retaining capacity at this time. The same freezing temperature is able to remove as ice a much smaller percentage of water during the period of greatest resistance than when the tissue is more readily killed by cold. This smaller portion removed as ice would mean less departure from the normal water relationships in the tissue. It may be that it is necessary to remove as ice a rather definite percentage of the total water present before serious injury or death of the protoplasm results. Under such a condition the shoots in the winter could withstand a much lower temperature before this minimum percentage would be reached.

Too much emphasis should not be placed on the percentage of water unfrozen without a discussion of the percentage of total water. Numerous investigators (**1, 7, 23, 35, 55**) have pointed out that hardiness is associated with a low moisture content. Some authors (**22, 48**) have contended that hardy varieties can be separated from tender ones by their lower moisture content. Such is not the case with the apple shoots used here, although there is evidence that the degree of resistance to cold is related to the water content of the tissue. This relationship is readily seen from an inspection of the percentage water curves in figures 4 and 7. From summer to winter there is a marked decrease in the moisture content of the shoots. During the winter the percentage of water reaches a low level that remains quite constant until spring, when it rises very abruptly. It is obvious that when the shoots were least resistant the moisture content was high, while during the period of great resistance to cold the percentage was low.

A comparison of the two years shows that the percentage of water in the shoots was higher during the winter of 1931–32 than in the following year. A lower percentage of unfrozen water is also observable in 1931–32. The fact that the two years differ with respect to the position of their curves is probably correctly explained by the comparatively warm, moist autumn of 1931, in which some woody plants blossomed in later fall and many exhibited a renewal of growth at this time. A glance at the temperature and rainfall charts (figs. 5, 6, 8, 9) shows the difference between the two autumn seasons.

From past experience with winter injury to fruit trees it is known that the conditions of 1931 were not conducive to a thorough hardening as contrasted to the weather of 1932. DEXTER (**5**) and TUMANOV (**57**) have found recently that conditions favoring food accumulation are generally favorable for the hardening of plants. The season of 1931 was not especially favorable for the accumulation of foods as compared with the following autumn. It might be assumed, then, that the shoots were less hardy during this year than during the following year; an assumption that is supported by the smaller percentage of unfrozen water in the shoots.

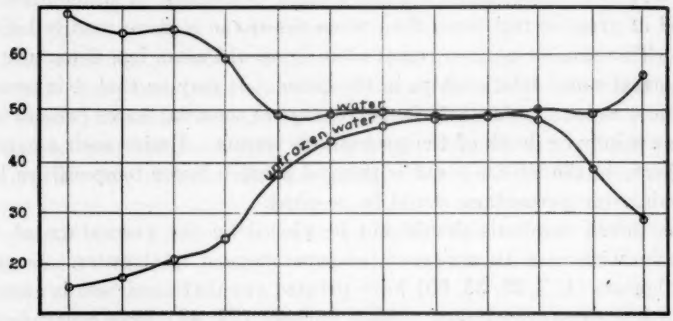

FIG. 7. Percentage of total and unfrozen water in nursery shoots, 1932–33.

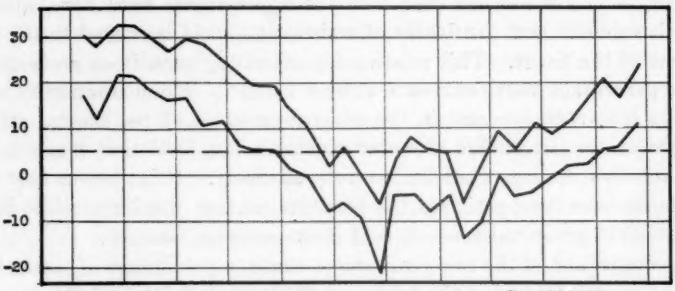

FIG. 8. Maximum and minimum temperatures, °C., 1932–33.

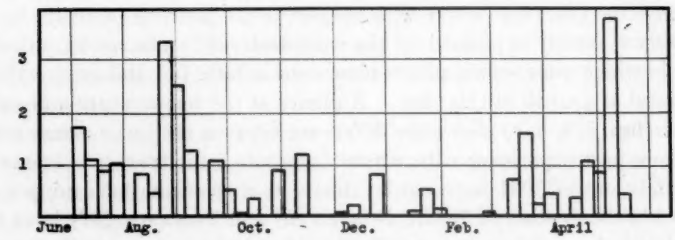

FIG. 9. Precipitation in inches, 1932–33.

Another relationship between the course of the curves for unfrozen water and climatic conditions is suggested by an investigation by HARVEY (17). In a study of elm seedlings he determined the threshold value for hardening to be about 5° C., when the exposure was continuous. Little or no hardening was observable at 10° C. In figures 5 and 8 it may be noted that the 5° line intersects the maximum temperature curves in the spring of both years at about the same time the drop in unfrozen water occurs. The influence of a warm temperature on the ability to retain water unfrozen was pointed out in a previous section and those results are apparently substantiated by a similar decrease observable here.

Discussion

In attempting to separate apple varieties into an order of hardiness many factors must be considered. First, the order of hardiness probably varies from time to time, with no variety maintaining the same relative position throughout the year. Not only is there a variation between varieties but the shoots of the same variety also respond differently to the same freezing temperature. Different degrees of injury through cold may occur without death resulting to the entire shoot. It is only when a sufficiently large portion of the living tissue is killed that recovery becomes impossible. The magnitude of this portion may vary among varieties and from shoot to shoot within a variety.

Again there is a difference in the same variety brought about by the external environment. The hardiness of a tree will vary with climatic and other conditions quite independently of its inherent resistance to cold.

In spite of these possible sources of variations, hardy varieties survive winters that kill the more tender sorts. It is this constancy of behavior that suggests some inherent difference in the hardiness of the varieties.

To test this inherent difference, by measuring the capacity to retain water unfrozen, is complicated by the factors mentioned above, plus the lack of information as to the necessity of this capacity in survival. The rate of freezing and the rate of thawing have both been shown to have an influence on the injury resulting from low temperatures. Neither of these measurably affect the quantity of water frozen. There are probably other factors influencing the amount of injury without appreciably altering the quantity of water frozen in the tissue. It is these additional influences that demand caution against placing too much emphasis upon capacity to retain water against freezing as the major factor in surviving cold. Nevertheless the importance of this capacity is evidenced again and again in the study presented here. This is especially noticeable in the effect of period of exposure to freezing temperatures, in the effect of a preceding warm period, in the comparisons at $-5°$ and $-20°$ C., and in the general course of unfrozen water throughout the year.

The attempts to separate the hardy from the tender varieties using unfrozen water as a basis were futile, with perhaps the one exception of the comparison made at $-5°$ and $-20°$ C. In this case the tender varieties, taken as a group, exhibited a greater loss of water in formation of ice between the two temperatures than did hardy varieties. Although the data indicate that such a procedure might serve for placing a variety into a hardy or tender class, more evidence is necessary to substantiate this point before a positive assertion can be made.

Summary

1. Unfrozen water measurements were made on apple shoots by the heat-of-fusion method at monthly intervals throughout the year.

2. Some evidence was obtained to show that the freezing process in apple shoots was partially reversible, resembling the behavior of an inelastic gel in this respect.

3. In general, the data supported the hypothesis that the capacity to retain water against freezing is associated with winter hardiness of apple shoots.

4. A statistical analysis of the data indicated that varieties differ in their capacity to retain water against a freezing temperature of $-20°$ C. Nevertheless it was impossible on this basis to separate the varieties into a hardy and a tender class.

Grateful acknowledgment is extended to Professors B. S. PICKETT, T. J. MANEY, and W. E. LOOMIS for suggestions during the course of this investigation.

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BIOCHEMICAL METHODS FOR THE STUDY OF NITROGEN METABOLISM IN PLANTS[1]

F. S. Orcutt and P. W. Wilson

(WITH TWO FIGURES)

Introduction

The analytical difficulties encountered in attempts to study the nitrogen metabolism of plants during the course of their growing and fruiting periods give rise to results from which it is hard to draw signficant conclusions concerning that metabolism. Chief among these is the inability of the analyst to separate and identify the various constituents which occur in the plant at the time of harvest without irreversibly altering the chemical nature of many of them before they can be determined. The lability of many compounds concerned in plant metabolism, combined with the drastic action of the reagents in the comparatively crude methods used, make it difficult to construct accurate nitrogen balances. It is quite probable that a thorough understanding of plant nitrogen metabolism will be greatly facilitated by the construction of such nitrogen balances.

The preparation, alone, of plant juice for analysis of nitrogen constituents has been the object of a number of investigations (**1, 4, 19**), but at the present time there is no generally used or accepted method. This is partially due to varying constituents in different types of tissue, but it is more directly due to the inadequacy of these methods for giving extracts or juices which can be readily analyzed. The objective of this paper is to present and suggest modifications of these various methods that give preparations which may be analyzed to give nitrogen balances as they probably occur in the plant. While these methods were developed specifically for the analysis of soybeans, they may be readily adapted to other types of tissue as well.

Investigation

Separation of water soluble from insoluble constituents

Extraction.—Prior to 1922 much of the work on the soluble nitrogen constituents was carried out on dried plant material. It is generally recognized now, however, that drying, freezing, storage in alcohol, and autoclaving all alter the form in which the nitrogen is found. Fresh material, therefore, is a necessity for nitrogen studies.

[1] Herman Frasch Foundation in Agricultural Chemistry, Paper no. 116. Contribution from the Departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

Extraction of soluble material with either hot or cold water or alcohol (1) is objectionable in that for quantitative extraction the final volume is twenty times that of the original sample. The resulting solution is so dilute that concentration under diminished pressure is necessary; that is, it is both laborious for numerous samples and it tends to bring about decomposition such as aminolysis.

Where the proper facilities are available, pressing out the sap of fresh tissue offers a much easier and a standard manner of preparing a solution of the soluble plant constituents. Numerous studies have been made on plant saps; the work of GREATHOUSE (5), MEYER (12), PETTINGER (13), and SAYRE and MORRIS (14, 15) are examples of several applications of this method. The method involves no addition of water, so that quantitative extraction is unnecessary; the results are expressed as concentration in the sap, which may be converted to dry plant weight basis if desired.

SAYRE and MORRIS found the determination of sugars as accurate as alcoholic extraction and that the concentration of sugars was unaffected by the size of sample, pressure, or preliminary treatment. The cage described by MEYER (12) for holding the tissue under the press, has several distinct advantages over the usual type, since it allows a more complete collection of the juice by less waste in containers, and a more thorough draining of the pulp pad. With this cage about 80 to 85 per cent. of the total water in the soybeans can be recovered, which is the same range of recovery that SAYRE and MORRIS (14) obtained with corn. This cage will conveniently accommodate from 100 to 150 gm. of plant material; and the load may be brought to 3000 lb. per square inch under a Carver laboratory press. Pressures up to 25,000 lb. per square inch may be obtained with a small Carver cage which accommodates 25 gm. of fresh material.

When soybean tissue was ground in a Nixtamal mill and then pressed it was found that the soybean sap coming from the press showed:

1. The concentration of total nitrogen is the same in sap from each cage under maximum load.

2. No difference was found in percentage of nitrogen in the first three-quarters and the last quarter of the sap drawn from the large cage.

This indicates that grinding in the Nixtamal mill before pressing mascerated the tissue sufficiently to make nitrogen concentration of the sap independent of the last juice to be squeezed from the pulp.

It is usually considered necessary to rupture the cell with some preliminary treatment such as application of ether. When 3000 lb. pressure is used, however, it was found advisable not to use ether because: (1) Ether carries over lipids into the water solution. (2) Its use did not increase the total nitrogen content of the sap. It may, therefore, be considered that a

sufficient number of cells were broken in the mill and in the press to give a representative sample of the sap.

The possibility may be raised of certain forms of nitrogen being adsorbed on the tissue remaining in the press in contradistinction to other forms of nitrogen freely expressed. It does not seem probable, however, that even if such preferential adsorption takes place that it could significantly alter the percentages in the representative sample of sap drawn.

PRECIPITATION OF COLLOIDS.—The next step in the preparation of the sap is to precipitate the chlorophyll and other colloids, and easily coagulable proteins. The most common means for this purpose are alcohol, heat, and acid. It is not desirable to use alcohol as a precipitant because of the necessity of completely removing it for the alpha amino nitrogen determination. This objection does not apply to heat, and the latter is also valuable in destroying enzymes.

Acids are quite generally used (3) to aid the precipitation by bringing the pH nearer to the iso-electric point of the proteins—assumed to be in the range of pH 4 to 5. Little attempt has been made in previous work, however, to accurately control the pH. HILLER and VAN SLYKE (6) recommend the use of tungstic acid. Tri-chloracetic and acetic acids have been used to some extent with fair success.

In table I are given data concerned with the reduction in total nitrogen by acid treatment. The pH of the soybean sap was approximately 5.8; the pH of other samples was adjusted with the glass electrode by the addition of tri-chloracetic acid. Each sample was heated, cooled in a refrigerator, and filtered. Total nitrogen was then determined on the clear filtrate.

TABLE I

REDUCTION IN TOTAL SOLUBLE NITROGEN IN SOYBEAN SAP BY HEATING AT VARIOUS LEVELS OF PH

TREATMENT OF SAP	TOTAL N	DECREASE IN N
	mg./cc.	%
pH 5.8 boiled 30 sec.*	1.28	0
pH 5.0 " " "	1.18	7.8
pH 4.5 " " "	1.16	9.4
pH 4.0 " " "	1.15	10.1
pH 4.0 boiled 5 min.	1.15	10.1
pH 3.5 " 30 sec.	1.15	10.1
pH 3.0 " " "	1.13	11.7

* Sap without acid; tri-chloracetic acid added to others.

The amount of protein precipitated is distinctly higher when tri-chloracetic acid is added. Boiling for 5 minutes has no further effect except to decompose the acid to chloroform and carbon dioxide.

Additional data on the effect of heat and acid on the total nitrogen of sap are shown in table II. After heating, samples were cooled and filtered as before.

TABLE II

REDUCTION IN TOTAL SOLUBLE NITROGEN OF SOYBEAN SAP BY TREATMENT WITH CCl_3COOH AND HEAT

SAMPLE	FROM THE PRESS*	HEATED TO 70° C.	BOILED 5 MIN. WITH CCl_3COOH AT pH 3	DECREASE IN N FROM SAP HEATED TO 70° C.
	mg. N/cc.	*mg. N/cc.*	*mg. N/cc.*	%
I.				
Tops	.....	3.21	2.93	9
Roots	1.13	0.69	0.62	10
II.				
Tops	3.19	1.85	1.69	9
Roots	0.59	0.38	0.34	10

* Includes chlorophyll, colloids, etc.

Further treatment of the boiled sample of tops with 25 per cent. alcohol gave a total reduction (from the sample heated to 70° C.) of 11 per cent.; 50 per cent. alcohol gave 18 per cent. reduction.

It is concluded, therefore, that the reagents commonly used for precipitation of highly organized nitrogenous compounds from plant sap, *viz.*, acids as well as alcohol, are only relative in their quantitative action, *i.e.*, the amount of precipitation is dependent upon the quantity of reagent added and does not give a solution free from peptides, and more highly organized forms of nitrogen compounds.

The heating of the plant juice to 70° C., however, easily coagulates the colloidal material and inactivates the enzymes without the further loss of soluble nitrogen. It is possible that this short heat treatment may have a slight hydrolytic action. After heating, the juice is cooled immediately and chilled in the refrigerator to complete precipitation. The flocculent precipitate is easily filtered on a paper pulp pad in a small Büchner funnel with slight suction. The filtrate, then, is a clear solution of undiluted plant juice.

HYDROLYSIS OF SOLUBLE CONDENSED FORMS OF NITROGEN

Consideration of the data in the preceding section on the preparation of sap for analysis makes it apparent that procedures such as treatment with acid or alcohol give little information as to the chemical constitution of the nitrogen fraction. Interest in the nitrogen metabolism is ordinarily con-

fined more to the type of compounds present rather than to the degree to which they are condensed. The soluble condensed forms are probably as important in the *active* metabolism of the plant as are the simple free amino acids. Instead of trying to free the solution of these condensed forms, a preferable procedure might be to hydrolyze and determine them with the other amino acids.

Acid hydrolysis, with either 20 per cent. HCl or H_2SO_4 requires long heating—12 to 24 hours. The acid hydrolysis of practically all protein gives rise to humin formation by condensation of tryptophane, ammonia, and other amino acids, with aldehydic compounds such as glucose. In some cases the amount of humin formed is small enough not to introduce any appreciable error in balancing the various nitrogen determinations. Plants in general, and soybeans in particular, give rise to 10 to 15 per cent. humin nitrogen—that is, 10 to 15 per cent. of the total soluble nitrogen condenses into humin.

The chemical reactions which take place during humin formation are generally thought to be oxidative in nature. It is possible, therefore, that if the solution during hydrolysis were sufficiently reduced these reactions would not be favored. Two attempts have been recorded in the literature to avoid excessive humin formation in the acid solution by the addition of reducing agents. HLASIWETZ and HABERMANN (7) first used stannous chloride in 1873 to prevent humin formation in casein hydrolysis. In 1929 SULLIVAN (17) reported the successful checking of humin formation in the same hydrolysis with titanous chloride. These chlorides were tried on soybean sap but showed no retardation of the humin formation during hydrolysis. Other reducing agents such as $Na_2S_2O_3$, zinc amalgam, HI, and thio-glycolic acid were also tried without success. Sodium bisulphite was found to decrease the amount of humin formed, so that a study was made to investigate the possible use of this reducing agent.

TESTING HYDROLYSIS IN PRESENCE OF BISULPHITE.—(Amide determination). The hydrolysis of amide nitrogen occurs in the general hydrolysis, and may be taken as a *first* criterion of whether any hydrolyzing action takes place under a given set of conditions. Most methods of amide determination are based on the method of SACHSSE (18). VICKERY (22) showed that sulphuric acid is preferable to hydrochloric because of a reducing action the latter may have on nitrates in the presence of reducing substances. The procedure generally followed (2, 18) is hydrolysis with 10 per cent. sulphuric acid for 2½ hours followed by distillation from the neutralized solution with an excess of sodium carbonate. More recently SESSIONS and SHIVE (16) aerate the ammonia out of solution. This latter method offers convenience in running numerous samples.

It was found that somewhat erratic results are obtained with sulphuric acid hydrolysis if refluxing over an open flame or sand bath is used. It was found that this could be completely avoided by hydrolyzing in a steamer for 2⅓ to 3 hours, *i.e.*, where a perfectly uniform heat treatment could be maintained. Recovery of amide nitrogen from asparagine by this procedure consistently gave recoveries of 99 per cent. Subsequent hydrolyses, therefore, were made in a steamer with the samples in 50-cc. bottles covered with bakelite caps (packing removed). Use of these caps eliminates the possible extraction of nitrogen from rubber stoppers or corks as is often encountered.

It was noticed that sap prepared by boiling had a greater tendency to form humin than when it had been heated to only 70° C. It is possible that much of the preliminary condensation goes on during this previous boiling. Therefore, saps in the subsequent work were prepared by heating to only 70° C.

Comparative hydrolyses of amide nitrogen were made with sulphuric acid and sodium bisulphite as the hydrolytic agents. At first, it was thought that some combination of the two would accomplish the hydrolysis and prevent humin formation. In a large number of samples, however, it was found that the best hydrolysis without humin was with bisulphite alone. In table III are given the recoveries of amide nitrogen in asparagin added to soybean sap as well as recoveries from pure solution.

TABLE III

RECOVERIES OF AMIDE NITROGEN (ASPARAGIN)*

RECOVERY FROM	REAGENT AND CONCENTRATION	NUMBER OF SAMPLES	HOURS IN STEAMER	PERCENTAGE RECOVERY OF AMIDE N
			hours	%
Pure solution	10% $NaHSO_3$	30	6	95 to 97
" "	10% $NaHSO_3$	4	3	85
" "	10% H_2SO_4	30	3	99 to 100
Soybean sap	10% $NaHSO_3$	55	6	95 to 97
" "	20% $NaHSO_3$	12	3	95 to 97
" "	10% H_2SO_4	30	3	99 to 100
Soybean sap	10% $NaHSO_3$ plus 10% H_2SO_4	6	3 to 6	75 to 85
" "	5% H_2SO_4	4	3	87
" "	10% $NaHSO_3$ plus 3% H_2SO_4	6	3 to 6	31 to 51

* 3.5 to 4 mg. amide N added to 10 to 20 cc. of sap containing from 0.5 to 2 mg. N per cc.

The pH of sap plus 10 per cent. sulphuric acid is approximately zero, in contrast to a pH of 4 for the bisulphite. When the latter is acidified to the pH range of 0 to 2, the recovery is very poor. The hydrolysis of amide nitrogen therefore is apparently not dependent particularly upon pH. When sulphuric acid is added, Na_2SO_4 is probably formed and H_2SO_3 liberated. The latter decomposes into SO_2 and water, and the SO_2 is boiled off. When no sulphuric acid is added and bisulphite is the only reagent, the pH is approximately 4; at this pH apparently enough sulphurous acid remains in solution to accomplish the hydrolysis with no trouble from humin formation. It is possible that the function of the bisulphite is the formation of bisulphite addition compounds with aldehydic groups as in glucose so that such groups are not free to condense with nitrogen-bearing groups.

When 20 per cent. bisulphite is used instead of 10 per cent., the hydrolysis takes place in 3 hours as with 10 per cent. sulphuric acid. This might be expected on the grounds that 20 per cent. bisulphite is equivalent to approximately the same amount of acid as is contained in 10 per cent. sulphuric acid. This shorter time of hydrolysis (3 hours with 20 per cent. bisulphite) gives much more constant liberation of ammonia from sap than does 6 hours, and agrees closely with the sulphuric acid hydrolysis. An example of such a comparison is given in table IV. No humin was formed in samples to which was added only $NaHSO_3$.

TABLE IV

NITROGEN LIBERATED FROM SOYBEAN SAP BY VARIOUS TREATMENTS FOR AMIDE HYDROLYSIS

TREATMENT	TIME OF HYDROLYSIS	MG. OF N	PERCENTAGE OF TOTAL N
	hours	*mg.*	%
15 cc. of sap			
plus 20% $NaHSO_3$	3	3.41	17.2
plus 10% H_2SO_4	3	3.41	17.2
plus 20% $NaHSO_3$	6	4.08	20.6
plus 10% $NaHSO_3$	6	3.65	18.5
plus 20% H_2SO_4	6	3.87	19.6

The additional ammonia formed during the longer periods of hydrolysis probably came from amide nitrogen tied up in peptide; this will be shown more clearly in a later section. It may also have arisen from deamination.

It was found that the bisulphite must be removed from solution before alpha amino nitrogen can be determined. The presence of bisulphite causes a large and inconsistent variation in the blank determination on reagents. At first it was removed by precipitation as the calcium salt by addition of calcium chloride ($CaSO_3$ has a solubility of only $0.004^{18°}$ gm. per 100 cc.).

Upon neutralization of the HCl set free, the clear filtrate gives no increase over the normal reagent "blank." It was feared, however, that the voluminous precipitate formed would carry nitrogen compounds down with it.

Aerating out the sulphite as SO_2 was tried as an alternative to precipitation. By addition of 15 per cent. H_2SO_4 to the solution of 20 per cent. $NaHSO_3$ after hydrolysis the sulphite can be removed quantitatively by *rapid* aeration for 4 to 5 minutes (full suction pump). If less than 15 per cent. H_2SO_4 is used, traces of sulphite are often left in solution and cause a small addition to the "blank."

The H_2SO_4 remaining in the solution from the removal of SO_2 may be neutralized to phenolphthalein with 10 N alkali. Two or three drops of alkali are added in excess and the amide nitrogen as ammonia is rapidly aerated into 56th normal acid. The sap is so well buffered that the addition of sodium hydroxide does not carry the pH of the solution beyond the range of 10. There are enough salts present in the solution so that an air flow of 1500 cc. per minute gives a quantitative collection of all the ammonia in the acid in four hours. (A trap containing glass wool is used between the tubes containing alkali and acid.)

TESTING THE BISULPHITE METHOD FOR PEPTIDE HYDROLYSIS.—It has been found that 20 per cent. sodium bisulphite gives hydrolysis of amides comparable to the standard sulphuric acid method without giving rise to the objectionable humin formation. The question now arises whether bisulphite is effective in peptide hydrolysis. To test this it is necessary to carry out the hydrolysis on some peptide-containing compound which will not give rise to humin when hydrolyzed with sulphuric acid. Table V shows the results of the hydrolysis of peptone by sulphuric acid and sodium bisulphite.

The bisulphite had practically no effect on the hydrolysis of peptone and can therefore be considered specific for amide in contrast to peptide linkages.

TABLE V

HYDROLYSIS OF 1 PER CENT. COMMERCIAL DIFCO PEPTONE SOLUTION BY $NaHSO_3$ AND H_2SO_4

TREATMENT	TIME OF HYDROLYSIS	α NH_2 – N IN PERCENTAGE OF TOTAL N
	hours	%
No hydrolysis	0	21
10% H_2SO_4	3	53
20% "	3	58
" "	5	62
" "	7	63
20% $NaHSO_3$	3	23
" "	7	23

ENZYME HYDROLYSIS.—Since bisulphite was found to have no hydrolyzing action on peptide linkages, it was necessary to find some agent which would bring about this part of the hydrolysis without causing humin to form also. It was thought possible that proteolytic enzymes should fulfill this requirement if complete hydrolysis could be obtained.

JOHNSON (8, 9) has described a mixture of enzymes obtained by a water extraction of *Aspergillus parasiticus.*[2] The enzyme solution contains at least one proteinase, one carboxy-polypeptidase, two amino-polypeptidases, and one di-peptidase. It is a fairly complete enzyme mixture in that proteins should be degradated completely to amino acids. Its pH optimum is 7 to 7.5. Peptides such as glycyl-glycine, leucyl-glycine, tri-glycine, etc., have been shown by JOHNSON and PETERSON (10) to be hydrolyzed completely in 4 to 5 hours at 40° C. They have recently shown that a racemic mixture of peptides will be hydrolyzed only to the extent of those corresponding to the natural products in optical activity. Table VI shows the extent of hydrolysis of peptone with this enzyme solution.

Hydrolysis of the peptone was slow and gave only half as much alpha amino nitrogen as did the sulphuric acid hydrolysis. It is possible that the amino acids may have become racemized during the manufacture of the

[2] *Preparation of Enzyme Solution* (8). *Aspergillus parasiticus* or *Aspergillus wentii* (the latter is more easily grown and gives as high a concentration of enzymes) is grown by seeding skim milk containing 5 per cent. glucose with mold spores, and incubating 6 to 8 days at 30° C. When the pad shows heavy sporulation and begins to crack and pull away from the edges of the container it is separated from the medium, ground in a mill, and extracted with water. If the enzyme is not to be used immediately, the pad can be preserved by freezing without appreciable loss of enzyme activity. Drying, however, tends to destroy the enzymes. For extraction, enough water is added so that the mixture contains approximately 10 per cent. dry matter. The pH is roughly adjusted to the range of 7.0 to 7.5 by the addition of sodium carbonate. Toluene is added and the mixture allowed to stand four to six hours at room temperature. The suspension is then filtered through "sail cloth" under vacuum. The pad which forms on the cloth acts as an effective filter and gives a clear filtrate. The enzymes are precipitated from this crude water solution with one volume of acetone. After centrifuging and pouring off the clear liquid, one-quarter of a volume of water is added, shaken up with the precipitate, and centrifuged again. The clear solution (I) is saved and another quarter volume of water shaken up with the precipitate. After centrifuging, the clear liquid (II) is mixed with (I). This solution is preserved under toluene in the refrigerator. Tyrosinase activity tends to blacken the solution on standing; this may be considerably reduced by displacing with hydrogen the air in and over the solution. This darkening of the solution does not affect the proteolytic potency, however. The solution is relatively low in nitrogen, and gives, therefore, only a very small correction factor in the various determinations.

Since this paper has gone to press, evidence has been obtained by PETERSON *et al.* that it is necessary to freeze the mold tissue before extraction in order to inactivate arginase which may be present. They also find that drying at room temperature destroys this enzyme and apparently leaves the proteolytic enzymes almost as active as in wet tissues.

TABLE VI

HYDROLYSIS* OF PEPTONE BY ENZYME SOLUTION

TREATMENT: 15 CC. OF 1 PER CENT. PEPTONE SOLUTION PLUS 2 CC. OF ENZYME SOLUTION

TIME	AT pH 7.3 α-NH_2-N IN PERCENTAGE OF TOTAL NITROGEN	TIME	AT pH 6.2 α-NH_2-N IN PERCENTAGE OF TOTAL
hours	%	*hours*	%
0.	21.0	0.	21.0
5.5	29.6	5.0	27.5
22.5	34.5	27.0	37.3
46.5	40.7	52.0	43.3
—	—	70.0	43.6

* Hydrolysis with 20 per cent. H_2SO_4 for 6.5 hours gave 62.5 per cent. of the total nitrogen in the α-amino form.

peptone and therefore gave reduced (approximately 50 per cent.) hydrolysis with the enzyme.

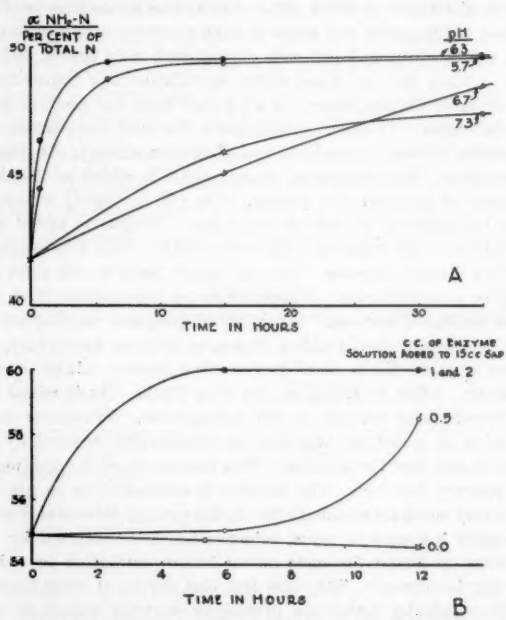

FIG. 1. *A*, action of enzyme solution on soybean sap at different pH levels; *B*, comparison of different quantities of enzyme solution in hydrolysis of soybean sap.

Figure 1A shows the hydrolysis of peptide linkages in soybean sap by enzymes, varying the pH and time. For some reason which is not apparent, incubation of sap at 40° C. in the presence of the enzyme (and under toluene) causes a black flocculent precipitate to form at pH 7 to 7.5 soon after incubation commences. At pH 6.3 the precipitate is first noticed at about 5 hours, but never reaches the amount found at pH 7.3. At pH 5.7 only a slight precipitate is noticed after 50 hours. In figure 1A it is seen that at pH 5.7 to 6.3 the maximum hydrolysis was obtained between 6 and 15 hours. Six hours is the time reported by JOHNSON (**10**) for pure dipeptides in the optimum range of 7 to 7.5. In soybean sap the hydrolysis appears to take place faster at pH 5.7 to 6.3 than at the higher values. This is coincidental with the absence of a precipitate at pH 5.7 after 15 hours, and is much more convenient of operation since the pH of soybean sap ranges from 5.5 to 5.8, so that no pH adjustment needs to be made.

The *quantity-time* relation of the enzyme solution is shown in figure 1B. At least 1 cc. of enzyme solution was necessary to hydrolyze the peptide linkages in 15 cc. of sap.

The enzyme hydrolysis seems to have little if any significant hydrolyzing action on amides as shown in typical data given in table VII. There was a slight increase in ammonia with enzyme hydrolysis but this was small compared with the total amount of amide present.

TABLE VII

HYDROLYSIS OF AMIDE BY ENZYME AND BISULPHITE

TREATMENT	α NH_2-N AFTER TREATMENT IN PERCENTAGE OF TOTAL N	
	SAP 1	SAP 2
	%	%
No hydrolysis	1.1	1.6
Enzyme hydrolysis	2.7	3.4
20% $NaHSO_3$	21.1	21.1

It appears, then, that the enzyme solution hydrolyzes the peptide linkages in soybean sap according to a typical curve obtained with synthetic peptides —one which rises rapidly and approaches the maximum values asymptotically. It does not, however, appreciably attack the amide linkages, so that the enzyme hydrolysis must be followed by a 20 per cent. sodium bisulphite hydrolysis for 3 hours in the steamer. The bisulphite does not attack the peptide linkages, as shown in a previous section, so that both enzyme and bisulphite must be used to complete the hydrolysis.

The amount of peptide nitrogen in soybean sap heated to 70° C. is small, but is large enough to figure significantly in the nitrogen balance. This will be more apparent in a later section. The amount does not remain constant and was found to vary in the range of 5 to 15 per cent. of the total soluble nitrogen, depending upon what part of the plant is taken.

Comparison of nitrogen fractions with and without enzyme hydrolysis

To determine just how much difference might exist between nitrogen separation with and without enzyme hydrolysis, the determinations summarized in table VIII were made on 15-cc. samples of juice from soybean tops containing 2.18 mg. total nitrogen per cubic centimeter, and the juice of soybean roots containing 0.502 mg. of nitrogen per cubic centimeter.

The basic *amino* nitrogen is obtained by subtracting the alpha amino nitrogen, which is found in the filtrate after filtering the phosphotungstic acid precipitation (PTA), from the alpha amino nitrogen before the precipitation. The basic *non-amino* nitrogen is calculated by subtracting this basic amino nitrogen figure from the total basic nitrogen. The non-basic amino nitrogen is that determined in the PTA filtrate. "Other nitrogen" is determined by difference to make a total of 100 per cent. These fractions are calculated in this manner to make the nitrogen figures additive, *i.e.*, so one figure does not overlap with another one, and also to give a better idea as to the forms of nitrogen present.

Differences in the nitrogen fractions after enzyme hydrolysis indicate the hydrolysis of peptides or higher soluble forms. Approximately the same increase in amide nitrogen was obtained with 20 per cent. sulphuric acid hydrolysis as with enzyme hydrolysis, *viz.*, 32.6 in root sap instead of 31.6—an increase of approximately 4 per cent. over the figure in unhydrolyzed sap. With the acid hydrolysis, however, there was a loss of 12.6 per cent. of the alpha amino nitrogen in humin condensation.

It is interesting to note the liberation in both top and root sap of as much amide nitrogen as non-basic *amino* nitrogen, which may indicate that amino acids in the peptides, other than basic ones, were chiefly amides of dicarboxylic amino acids. Keyssner and Tauböck (**11**) recently pointed out that no amide nitrogen could be obtained from l-leucyl glutamin without first hydrolyzing the peptide linkage.

It is known (**3**) that part of the peptide nitrogen may be precipitated with phosphotungstic acid and some may not.

Reduction, after enzyme hydrolysis, occurred in basic *non-amino* nitrogen and "other nitrogen." Approximately one-half of the peptide nitrogen in the unhydrolyzed sap, then, was precipitated with phosphotungstic acid as basic nitrogen and one-half was not precipitated, and was therefore classified as "other nitrogen." By the complete hydrolytic action of the enzyme

TABLE VIII

COMPARISON OF NITROGEN FRACTIONS OF SOYBEAN SAP WITH AND WITHOUT ENZYME HYDROLYSIS
(ALL FIGURES IN PERCENTAGE OF TOTAL SOLUBLE NITROGEN)

FORM OF NITROGEN	NO HYDROLYSIS	AFTER ENZYME HYDROLYSIS	CHANGES AFTER ENZYME HYDROLYSIS
	%	%	%
Top sap			
NH_3 N	1.1	2.7	+1.6
Amide N	20.6	24.4	+3.8
Basic amino N	12.6	12.4	−0.2
Basic non-amino N	15.0	9.9	−5.1
Non-basic amino N	29.9	33.8	+3.9
"Other N"*	20.8	16.8	−4.0
Total	100.0	100.0	9.3
Root sap			
Amide N plus NH_3 N	27.9	31.6	+3.7
Basic amino N	12.7	14.7	+2.0
Basic non-amino N	8.8	3.8	−5.0
Non-basic amino N	39.5	43.2	+3.7
"Other N"*	11.1	6.7	−4.4
Total	100.0	100.0	9.4

* By difference.

solution the peptide nitrogen, amounting to 9.4 per cent. of the soluble nitrogen, was reclassified to give a truer balance of the forms of nitrogen present.

DETAILED PROCEDURE FOR PREPARATION AND ANALYSIS OF SOYBEAN SAP

PREPARATION.—The tissue is separated into the desired fractions (such as nodules, roots, stems, and leaves), cut up with scissors and ground in a Nixtamal mill. The pulp is pressed out in a Meyer cage on a Carver laboratory press at approximately 3000 lb. per square inch. When the material is not very fibrous the pulp is separated from the drain plate by one thickness of a 60-mesh "sail cloth" and a 20-mesh copper screen to hold the cloth from the plate perforations.

The sap is heated immediately to 70° C., cooled rapidly, and stored in the refrigerator overnight to complete precipitation. It is then filtered through a paper pulp pad in a 2-inch Büchner funnel. Enough of the sap is allowed to pass through the pad to displace the remaining water before

collection of the sample is begun. The clear filtrate is preserved under toluene in the refrigerator.

TOTAL NITROGEN.—The nitrogen content of the solution is determined on 2 to 4 cc. of clarified sap by the semi-micro aeration method of UMBREIT and WILSON (**20**).

HYDROLYSIS.—Two cubic centimeters of enzyme solution are added to 15 cc. of clarified sap in a 50-cc. bottle, protected from bacterial action by 1 cc. of toluene, the bottle covered with a Bakelite cap with packing removed, and incubated at 40° C. for 12 to 15 hours. Three grams of sodium bisulphite are then added to the bottle and the contents put in a steamer for 3 hours. (A white granular precipitate sometimes forms. This was shown to be nitrogen-free sulphite, probably calcium sulphite, which arises from soluble calcium in the sap.)

AMIDE NITROGEN.—After cooling, the solution is transferred to a large test-tube, 2.3 cc. of 20 N H_2SO_4 are added, and the solution rapidly aerated for 5 minutes to remove SO_2 (full suction pump). The excess acid is neutralized to phenolphthalein with 10 N alkali, and 0.2 cc. of 10 N alkali added past the end point, and the solution aerated 4 hours into 56th normal acid at the rate of 1500 cc. of air per minute. The pH is adequately controlled by the buffering capacity of the sap. Brom-cresol-green is an effective indicator to use with normal 56th reagents. The added enzyme increases the amide titration 0.1 mg. which generally amounts to 2 or 3 per cent. of the total amide figure.

ALPHA AMINO NITROGEN.—The alkaline solution is now acidified with acetic acid and the volume made to 25 cc. Five cubic centimeters are used for Van Slyke determinations.

BASIC NITROGEN.—Twenty cubic centimeters of the above solution are used for phosphotungstic acid precipitation by the semi-micro method of UMBREIT and WILSON (**21**).

ALPHA AMINO NITROGEN AFTER REMOVAL OF BASIC NITROGEN.—The acid PTA filtrate is neutralized to phenolphthalein with 10 N alkali, acidified with acetic acid and diluted to 30 cc. Ten cubic centimeters of this solution may be used for Van Slyke determinations.

NITRATE NITROGEN.—A correction of the total nitrogen figure for nitrate can be determined on the remaining 20 cc. of the PTA filtrate. The solution is made alkaline to phenolphthalein and enough alkali added in excess to make a concentration of approximately eighth normal. One gram of Devarda's alloy is added and the solution aerated over night at the rate of 1500 cc. per minute into normal 56th acid as in the amide determination. It was found that nitrate added to plant sap could be recovered quantitatively at this point in the procedure, so that treatment with bisulphite, etc., had no reducing action on the nitrate.

Figure 2 gives a graphic plan of the entire procedure for the preparation and analysis of soybean sap.

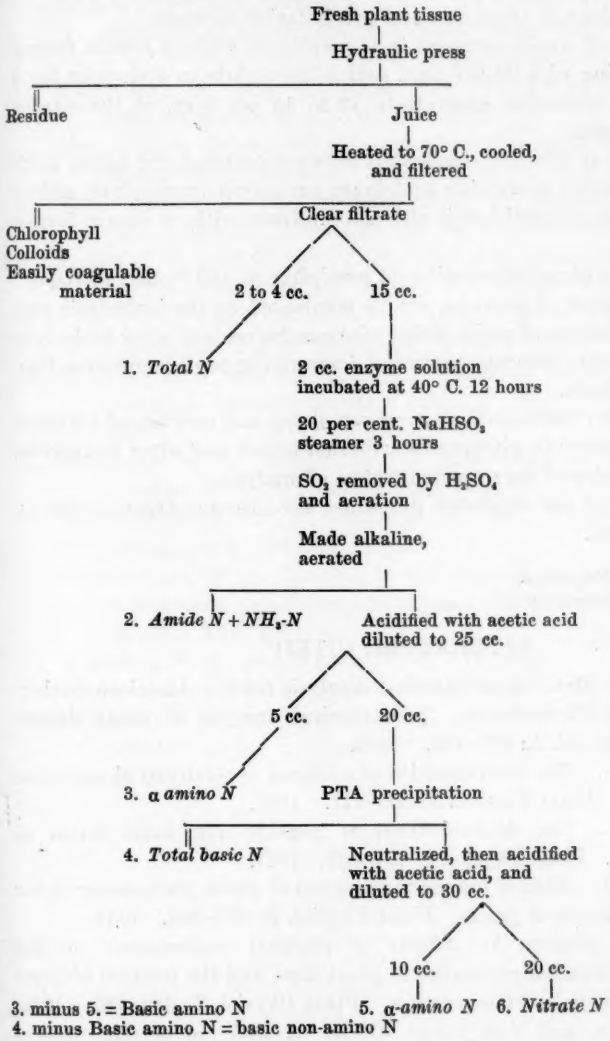
Fresh plant tissue
Hydraulic press
Residue
Juice
Heated to 70° C., cooled, and filtered
Clear filtrate
Chlorophyll
Colloids
Easily coagulable material
2 to 4 cc.
15 cc.
1. Total N
2 cc. enzyme solution incubated at 40° C. 12 hours
20 per cent. NaHSO3 steamer 3 hours
SO2 removed by H2SO4 and aeration
Made alkaline, aerated
2. Amide N + NH3-N
Acidified with acetic acid diluted to 25 cc.
5 cc.
20 cc.
3. α amino N
PTA precipitation
4. Total basic N
Neutralized, then acidified with acetic acid, and diluted to 30 cc.
10 cc.
20 cc.
5. α-amino N
6. Nitrate N
3. minus 5. = Basic amino N
4. minus Basic amino N = basic non-amino N

FIG. 2. Scheme of quantitative separation of soluble nitrogen constituents in plant tissue.

Summary

1. A method of preparing plant sap for analysis is suggested which eliminates the uncertainty of extraction and precipitation methods.

2. Hydrolysis of amide nitrogen is accomplished without humin formation by hydrolyzing with 20 per cent. sodium bisulphite in a steamer for 3 hours. (Humin formation amounts to 12 to 15 per cent. of the soluble nitrogen in soybeans.)

3. Hydrolysis of nitrogen compounds between proteins and amino acids by an enzyme solution containing proteinase, carboxy-polypeptidase, amino-polypeptidase, and di-peptidase is also accomplished without humin formation.

4. The error in phosphotungstic acid precipitation and "other nitrogen" owing to the presence of peptides, etc., is eliminated by the hydrolysis procedure. Determination of alpha amino nitrogen before and after hydrolysis may be used as a more accurate method of determining peptide nitrogen than precipitation methods.

5. Two nitrogen fractionations are given of top and root sap of soybeans showing the difference in nitrogen distribution before and after hydrolysis, to illustrate the value of the proposed system of analysis.

6. An outline of the suggested procedure for nitrogen fractionation in plant juice is given.

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TIME FACTOR IN UTILIZATION OF MINERAL NUTRIENTS BY HEMP

SISTER MARY ETIENNE TIBEAU

(WITH NINE FIGURES)

Introduction

Certain recent studies in mineral nutrition of plants have shown that the time of fertilizer application, in relation to the plant's ability to use nutrients, is as important as the balance among the nutrients themselves. Since it is also known that the physiology of the staminate and pistillate plants is in sharp contrast, especially in dioecious plants, the writer has undertaken to determine the physiological changes in hemp (*Cannabis sativa* L.) by varying the composition of various nutrient solutions, and the time of growth in these cultures, in order to test the effect of the treatment upon the ultimate sex expression.

Procedure

Hemp (*Cannabis sativa*) was chosen for this investigation because it shows marked sexual dimorphism and grows well under laboratory conditions. Seeds were planted in the greenhouse. When the seedlings were about 2 inches tall, they were removed from the soil and their roots were washed in distilled water. They were then transferred to 2-gallon jars containing white quartz sand which had been washed free from all solutes. Eight seedlings were planted in each of twenty-seven pots, on May 27, after being watered with distilled water. Four days later the pots were marked off in sets of three, and to each pot of each set there was added 500 cc. of a specially prepared nutrient solution. The plants were grown under continuous light for 12 days to stimulate vegetative development. As soon as they had attained sufficient growth, 6 plants of uniform size were selected from each set for observation.

All pots were kept constant in weight by the addition of distilled water, and 500 cc. of the nutrient solution was given as needed until 6 liters had been used.

The vegetative growth of the plants was rapid due to the length of the photoperiod. When the first photographs were taken on June 25, the plants being then 29 days old, very striking contrasts in growth and general appearance were apparent.

Knop's four-salt solution was used as the basic solution for supplying nutrients to the control plants. Three types of experiments, in which the basic nutrient solution was modified, were performed: (1) a high content

series in which the plants were supplied with a high content of the essential elements K, Mg, Ca, and N, respectively, added to a complete (Knop's) nutrient solution (table I); (2) a deficiency series in which the plants were supplied with a nutrient solution in which K, Mg, Ca, and N, respectively, were omitted from the complete nutrient solution (table II); and (3) a series in which the plants after first undergoing periods of starvation of a single element, were then supplied with a nutrient solution having a high content of this element.

TABLE I

COMPOSITION OF KNOP'S AND HIGH-CONTENT CULTURE SOLUTIONS GIVING WEIGHT IN GRAMS OF EACH SALT IN 14 LITERS OF SOLUTION

SALTS	CULTURE				
	KNOP'S	8K	8MG	8CA	8N
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
KNO_3	2.00	4.00	2.00	2.00	2.00
KH_2PO_4	2.00	4.00	2.00	2.00	2.00
$MgSO_4$	2.00	2.00	8.00	2.00	4.00
$Ca(NO_3)_2$	8.00	8.00	8.00	32.00	8.00
K_2SO_4	.....	9.10	.....	.....	.....
KCl	.....	5.74	.....	.....	.....
$MgCl_2$	.....	.....	6.34	.....	.....
$CaCl_2$	.....	.....	.....	21.64	.....
NH_4NO_3	.....	.....	.....	.....	32.80

TABLE II

COMPOSITION OF DEFICIENCY CULTURE SOLUTIONS GIVING WEIGHT IN GRAMS OF EACH SALT IN 14 LITERS OF SOLUTION

SALTS	CULTURE			
	−K	−MG	−CA	−N
	gm.	*gm.*	*gm.*	*gm.*
KNO_3	.....	2.00	2.00	.....
KH_2PO_4	.....	2.00	2.00	2.00
$MgSO_4$	2.00	.....	2.00	4.00
KCl	.....	.....	.....	1.48
$CaCl_2$	.....	.....	.....	5.50
$Ca(NO_3)_2$	8.00	8.00	.....	.....
$NaNO_3$	1.68	.....	8.32	.....
Na_2SO_4	.....	2.38	.....	.....
NaH_2PO_4	1.8	.....	.....	.....

Investigation

Experiments I and II

The plants supplied with a high-potassium (8K) solution showed an increase in growth compared with those supplied with Knop's solution, while the plants supplied with a potassium-deficient (−K) solution exhibited a retarded development (fig. 1). These differences in height, however, were

Fig. 1. Comparative growth of vegetative hemp in sand cultures variously treated with potassium. Age 29 days.

not maintained. At the end of 47 days, although the plants supplied with high-potassium (8K) solution were somewhat taller, plants supplied with Knop's solution had attained almost the same height; and the plants supplied with a potassium-deficient (−K) solution were lagging far behind,

Fig. 2. Comparative growth of vegetative hemp in sand cultures variously treated with magnesium. Age 29 days.

owing to the fact that potassium is abundantly utilized by the plant in the early stages of its growth (**2, 4**).

The plants supplied with a high-magnesium (8Mg) solution, at this time, were taller than those supplied with a magnesium-deficient solution (fig. 2), but the most striking contrast was in the color, the latter showing chlorosis (**9**).

The plants supplied with a high-calcium (8Ca) solution were shorter than any of the high-content series so far considered, yet they were dark green in color and looked healthy. The plants supplied with a calcium-deficient (– Ca) solution were stunted in growth, the eleventh node scarcely reaching the eighth node of the plants grown in a high-calcium (8Ca) solution (fig. 3).

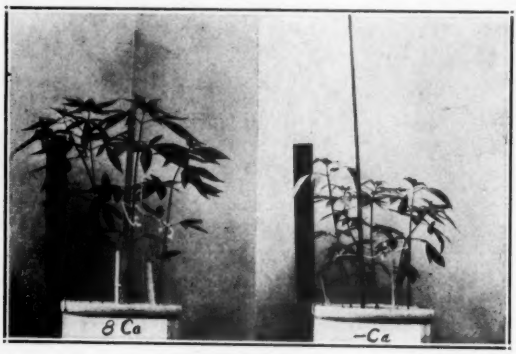

FIG. 3. Comparative growth of vegetative hemp in sand cultures variously treated with calcium. Age 29 days.

FIG. 4. Comparative growth of vegetative hemp in sand cultures variously treated with nitrogen. Age 29 days.

The plants supplied with a high-nitrogen (8N) solution and those supplied with a nitrogen-deficient (−N) solution showed by far the most striking contrasts (fig. 4). At this stage of growth, the best plant of the set supplied with a high-nitrogen (8N) solution was equal in development to the plants supplied with a high-potassium (8K) solution (fig. 1) and had produced an abundance of dark green foliage; but shortly after the plants were photographed brown spots covered the leaves, and they developed brown-rimmed shot-hole markings. In a short time the leaves rolled laterally and the whole plant showed signs of wilting. The marginal rolling up of the leaves may have been a means by which the plant checked excessive transpiration, since the weather was unusually hot. The plants supplied with a nitrogen-deficient solution were very small and of a pale yellowish-green color.

The height of hemp plants grown in high-content series of nutrient solution was in the order: 8K > 8Mg > 8Ca > 8N; and the height of those plants grown in the deficiency series of solutions was in the order: −Mg > −Ca > −K > −N.

Observations on the hemp plants were recorded weekly during the period of vegetative growth and the early reproductive stage (table III). In general appearance the plants supplied with the high-potassium (8K), Knop's, and high-calcium (8Ca) solutions were healthy looking and dark green, while the plants supplied with a high-magnesium (8Mg) solution were tall, and had long internodes, but were pale green. An excess of nitrogen produced a green leafy plant, but the nitrogen proved to be toxic.

The leaves of the plants supplied with a high-magnesium (8Mg) solution showed brown spot, shot hole with brown rim, and tip scorch; those supplied with a high-nitrogen (8N) solution, brown spot and shot hole with a brown leaf margin; while those plants supplied with potassium-deficient (−K) and the calcium-deficient (−Ca) solutions showed only brown spot, which JAMES (8) has designated as "coppering," and defined as a characteristic spot deficiency of potassium.

The plants supplied with a high-potassium (8K) solution produced the largest leaves. They had approximately fourteen times more leaf surface than the plants supplied with a nitrogen-deficient (−N) solution. The leaves of the latter plants were the smallest produced.

The most striking contrast of these two experiments was between the plants supplied with a high-nitrogen (8N) solution and those supplied with a nitrogen-deficient (−N) solution. The plants of the former series (8N) produced an abundance of dark green foliage, and all plants had begun to differentiate into females before they wilted and died; the latter series (−N) had small, pale green leaves, and all plants were males. Sex ratios

TABLE III

COMPARATIVE OBSERVATIONS ON HEMP PLANTED MAY 27, 1934, AND RECORDED JULY 18, 1934. X, SMALL AMOUNT; XX, SLIGHTLY MORE; XXX, MODERATE AMOUNT; XXXX, ABUNDANT

SOLUTION	GENERAL APPEARANCE	LEAVES	PROTEIN DISTRIBUTION IN STEM	STARCH DISTRIBUTION IN PLANT STEM		AV. HEIGHT OF PLANTS		SEX RATIOS	STEM TEXTURE
				MALE	FEMALE	♂	♀		
						cm.	*cm.*	%	
Knop	Healthy Dark green	Leaflet 12.5 × 2.2 cm.	Xylem—XX	Xylem—XX Pith—X Phloem—X	Xylem—XXX Pith—X Phloem—X	77.2	81.7	♀ 65 ♂ 35	Solid, herbaceous, fluted edge
8K	Healthy Dark green	Leaflet 13.6 × 2.5 cm. White pin-point spots on leaves	Xylem—XX	No male plant available	Xylem—XXX Phloem—X	86.5	78.6	♀ 73 ♂ 27	Hollow pith, woody, fluted edge
−K	Apical meristems pale Paler than 8K	Leaflet 9.2 × 1.6 cm. Brown spot on leaves Bleaching in vein islets	♂ Xylem—XX ♀ Xylem—XXXX	Xylem—XXX Phloem—XX Pith—X	Xylem—X Phloem—XX Pith—XXX	68.0	62.3	♀ 53 ♂ 47	Less woody, fluted edge, ♂ hollow, ♀ solid
8Mg	Plants tall with long internodes Pale green	Leaflet 10.2 × 1.4 cm. Brown spot on older leaves; white to yellow vein islets; tip scorch; shot hole with brown rim	♀ Xylem—XXX ♀ Cortex—XX ♂ Xylem—XX	Xylem—XXX Phloem—XX	Phloem—XX Xylem—XXX	108.5	95.4	♀ 40 ♂ 60	Fibrous, hollow pith, woody, fluted edge
−Mg	Stems grow smaller at top Chlorosis evident	Leaflet 11.5 × 1.7 cm. Yellow vein islets; white pin-point spots on leaves	♂ Xylem—XX ♀ Xylem—X	Xylem—XXXX Phloem—XX Pith—X	Xylem—XXXX Phloem—XXX Pith—X	79.6	74.0	♀ 53 ♂ 47	Hollow pith, ♀ herbaceous, fluted edge, ♂ woody
8Ca	Healthy Dark green	Leaflet 13 × 1.8 cm. Less abundant foliage Bleaching in vein islets	♂ Xylem—XX ♀ Xylem—X	Xylem—XXX Phloem—XX	Xylem—X Pith—X	101.9	86.3	♀ 33 ♂ 67	Fibrous, woody, hollow pith, fluted edge

TABLE III—(*Continued*)

Solution	General appearance	Leaves	Protein distribution in stem	Starch distribution in plant stem		Av. height of plant		Sex ratios	Stem texture
				Male	Female	♂	♀		
						cm.	*cm.*	%	
−Ca	Plants shorter than Knop's Pale green	Leaflet 10.1 × 1.7 cm. Brown spot and white pin-point spots on leaves	♀ Xylem—XXX ♂ Xylem—XX	Phloem—X	Phloem—X	73.1	76.1	♀ 57 ♂ 43	Fibrous, woody, hollow pith, fluted edge
8N	Abundant foliage	Leaflet 11.5 × 2 cm. Leaves rolled laterally; shot hole with brown rim; brown spot general	♀ Xylem—XXX Cortex—XX		Xylem—X Phloem—X		78.7	♀ 100	Herbaceous hollow pith, fluted edge
−N	Plants short and abnormal looking Pale to yellowish-green; red stems and petioles	Leaflet 6.3 × .9 cm. White pin-point spots general	♂ Xylem—XX	Xylem—XXX Phloem—X		98.3		♂ 100	Woody, hollow pith, fluted edge

(table III) in the other cultures ranged from 100 per cent. male plants in those that were supplied with a nitrogen-deficient (−N) solution to 100 per cent. female plants in those that were supplied with a high-nitrogen (8N) solution.

A group of plants grown in distilled water had the same characteristics as those supplied with a nitrogen-deficient solution: *viz.*, pale green, stunted growth, red stems, and a preponderance of males. It seems that in the absence of nitrogen the other essential nutrients are not utilized. Furthermore, the fact that the flowers of hemp (*Cannabis*) are sometimes perfect suggests that both sex potentialities are present, but only one is normally expressed. The sex of the plant would, then, seem to depend, in large measure, upon the presence or absence of certain nutritional factors (**10**). In the groups under consideration, the plants supplied with high-content and nitrogen-deficient solutions, the presence of nitrogen apparently was the factor which determined the female sex of the plants supplied with a high-nitrogen (8N) solution; and the absence of nitrogen may have been the factor which determined the male sex of the plants supplied with the nitrogen-deficient (−N) solution.

Experiment III

Experiment III was performed to determine the feeding capacity of the hemp plant: that is, to learn how long an essential element might be withheld from a plant without permanent injury; to what extent it would recover; and how rapidly such recovery would take place after a more or less prolonged period of starvation.

For this experiment four set of plants were used. The first set was designated as $-K_1$, $-Mg_1$, $-Ca_1$, and $-N_1$ and each pot was first supplied with two liters of a solution lacking the element indicated. The plants were grown in this solution 27 days. Following this each pot was supplied with a solution containing a high content of the same element. For example, in the case of the −K deficiency series, 2 liters of the −K solution were followed by 6 liters of 8K solution.

The plants of the second set were designated as $-K_2$, $-Mg_2$, $-Ca_2$, and $-N_2$, and each pot of the set was first supplied with 4 liters of a solution lacking the element indicated. The plants were grown in this culture for 44 days. Following this they were supplied with 4 liters of a solution containing a high content of the same element.

The plants of the third set were designated as $-K_3$, $-Mg_3$, $-Ca_3$, and $-N_3$, and each pot of the set was supplied with 6 liters of a solution lacking the element indicated. The plants were grown in this culture for 57 days. Following this the plants were then supplied with two liters of a solution containing a high content of the same element.

The plants of the fourth set were designated as $-K_4$, $-Mg_4$, $-Ca_4$, and $-N_4$, and each pot of the set was supplied with 8 liters of the solution lacking the element indicated.

On July 18, at the age of 27 days, as previously stated, the deficiency culture solutions were replaced by high content solutions of the essential elements previously lacking.

Two weeks later (fig. 5) there was a marked improvement in the appearance of the plants supplied with the 8 K solution. There was an increase

FIG. 5. Recovery of 6-weeks-old hemp plants after a 27-day potassium starvation period. $-K_4$ plants without potassium for 6 weeks; $-K_1$ plants without potassium until 27 days old; 8K plants supplied with potassium for 6 weeks.

in height, in size of the leaves, and in vigor of growth. The hemp was growing rapidly at this time owing to the length of the photoperiod (Mazda lights, 1000 watts per square yard 1 foot above the tops of plants were used daily from 6:00 P.M. to 11:00 P.M.). In two weeks the average height of the plants of the $-K_1$ group increased from 11.4 to 35.2 cm., while the plants of the $-K_4$ group, grown in a solution lacking potassium, increased from 13.5 to 30.2 cm. This shows a growth difference of 7.1 cm. or a daily increase of 0.5 cm. more for the plants of the $-K_1$ group than for those grown in the potassium-deficient (−K) solution.

In the magnesium groups the difference in height was not so significant, all of the plants having attained a growth almost equivalent to that of the control. Chlorosis, however, was evident in the plants grown in the magnesium-free solution. Recovery here was slow but at the end of 2 weeks the new growth at the apical meristem was darker green, and 4 days later the lower leaves showed the same recovery.

Recovery in the calcium groups was apparent in the leaves, which became darker green, and in the disappearance of white stripings which had been quite general.

A very striking recovery was made by the plants of the nitrogen-deficient ($-N_1$) group (fig. 6). After 27 days of nitrogen starvation, these plants,

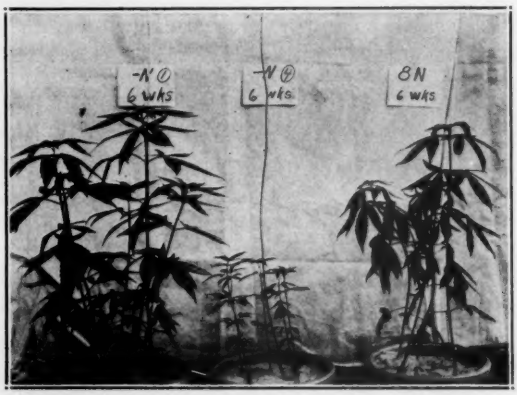

FIG. 6. Recovery of 6-weeks-old hemp plants after a 27-day nitrogen starvation period. $-N_4$ plants without nitrogen for 6 weeks; $-N_1$ plants without nitrogen until 27 days old; 8 N plants, with nitrogen for 6 weeks.

on receiving a high-nitrogen nutrient solution, were able to make rapid growth, increasing daily 1.1 cm. more in height than plants growing in a nitrogen-deficient solution. Recovery was also apparent in the leaves which, in the new growth, were much larger and dark green. The lower leaves recovered slightly but the new growth remained quite distinct from the old. The stems increased in diameter throughout their entire length and the red color was either masked by the chlorophyll or disappeared. This rapid recovery may have been due to the fact that the nitrogen-free solution used in this experiment contained potassium chloride and monopotassium phosphate (table II). Since, in the absence of nitrogen, potassium is not absorbed, it seems reasonable that as soon as the high-nitrogen (8N) nutrient was applied an abundance of potassium became available to the plant and it was utilized quickly.

After 44 days of potassium shortage, the nutrient solution of the $-K_2$ group was changed to a high potassium (8K) solution. In the 2 weeks which followed, the daily increase in growth was 1.27 cm. more than that of the plants grown in the potassium-free solution (fig. 7). At the same time a marked improvement was apparent in the appearance of the foliage and in the color of the plants. Likewise the plants of the $-K_3$ group were sup-

plied with a high-potassium nutrient after a starvation period of 58 days, after which they increased in height 0.6 cm. more daily than the plants grown in the potassium-free solution.

The daily increases in the height of the plants owing to the addition of K to the nutrient after various periods of shortage were as follows:

After 27 days of shortage	0.50 cm.
" 44 " " "	1.27 "
" 58 " " "	0.60 "

It is evident from these data that, for hemp, approximately 44 days of initial shortage gives optimum increase in vegetative growth. However, the final data show that while some of the plants in each initially deficient group were able to recover from the deficiency and attain a height equal to that of plants continuously supplied with potassium, yet the average height of the plants was considerably less.

Final observations on these plants showed that the plants grown in a high-potassium (8K) solution (fig. 7) were tall and had large leaves, but

FIG. 7. Recovery of 64-day-old hemp plants from K starvation. 8K plants supplied with high-potassium nutrient 64 days; $-K_1$ plants supplied with K-deficient nutrient 27 days and high-potassium 37 days; $-K_2$ plants supplied with potassium-free solution 44 days and high potassium 20 days; $-K_3$ plants supplied with potassium-free nutrient 58 days and high potassium 6 days; $-K_4$ plants without potassium.

meristematic activity had ceased. The plants of the $-K_1$ and the $-K_2$ groups were equally tall, in some cases even taller, and were still growing. The plants of the $-K_3$ group showed some improvement in growth but the leaves were still small and the color was notably paler green than those of the plants already described. The plants of the $-K_4$ group were stunted and the leaves were generally covered with brown spot.

Apparently potassium is necesary in the early life of the plant; and hence it seems reasonable to the writer that the greater the requirement for potassium, at least to a limited extent, the more rapidly it will be absorbed and utilized (**6**). The extreme heat and the lateness of the season prevented the continuation of the experiment to a point where potassium starvation would result in permanent injury and the plant would be unable to utilize any potassium.

Recovery of the plants in the magnesium groups was somewhat different from the others. In the $-Mg_1$ group of plants, from which magnesium had first been withheld for 27 days, no increase in growth attributable to magnesium was observed until 2 weeks after the high-magnesium (8Mg) nutrient solution had been supplied. The plants of the $-Mg_2$ group recovered more rapidly than those of the $-Mg_1$ group, but the plants of the $-Mg_3$ group were unable to recover from initial shortage of 58 days. The results of recovery expressed in daily increase in height of the plants owing to the addition of Mg to the nutrient after various periods of shortage are as follows:

After	27	days	of	shortage	.....	1.93	cm.	(4 wk. av.)
"	44	"	"	"	.....	1.08	"	(2 wk. av.)
"	58	"	"	"	.....	.....		

Since all of the plants of these groups attained considerable height, even those grown in magnesium-free solutions, it appears that magnesium has more to do with the actual life of the plant than with the vigor of its growth. Recovery in this case involves the production of chlorophyll. In general, the final data showed that the tips of the plants grown in the high-magnesium (8Mg) solution were dead and that there was some growth at the nodes. The leaves of the plants of the $-Mg_1$ group were dark green; the lower leaves of the plants of the $-Mg_2$ group were yellowish-white and dropping off, and the upper parts of the plants were green; while the plants of the $-Mg_3$ and $-Mg_4$ groups died.

Recovery in the calcium group (fig. 8) was more striking than in that of any group previously considered. In contrast with the potassium- and magnesium-deficient series, these plants were not only able to recover from the initial shortage (**3**), but they were able to make a better and a more rapid recovery the longer the calcium was withheld. It was observed in the final data (fig. 8) that both the plants grown in the high calcium (8Ca) and the calcium-deficient ($-Ca$) solutions were stunted, while the plants of the $-Ca_1$, $-Ca_2$ and $-Ca_3$ groups, from which calcium had been withheld for various periods, were taller and more vigorous.

The daily increases in growth of plants imputable to the addition of calcium after various periods of shortage were as follows:

FIG. 8. Recovery of 64-day-old hemp plants from calcium starvation. 8Ca plants supplied with high calcium nutrient 64 days; $-Ca_1$ plants supplied with calcium-free nutrient 27 days and high calcium solution 37 days; $-Ca_2$ plants supplied with calcium-free nutrient 44 days and high-calcium solution 20 days; $-Ca_3$ plants supplied with calcium-free nutrient 58 days and high calcium solution 6 days; $-Ca_4$ plants without calcium.

After	27	days	of	shortage	0.23	cm.
"	44	"	"	"	0.43	"
"	58	"	"	"	2.60	"

Since the plants grown in a calcium-free nutrient had practically ceased meristematic activity when the last data were taken, the results in terms of daily increase in height are as follows:

After	27	days	of	shortage	1.5	cm.
"	44	"	"	"	2.2	"
"	58	"	"	"	2.6	"

These data indicate that the limit for recovery from calcium starvation for this particular group of hemp plants is approximately 60 days, after which there is a cessation of meristematic activity followed by death of the apical meristems.

In the plants of the nitrogen group, those which had been grown in a high-nitrogen (8N) solution died early (7). The plants of the $-N_1$ group made a good recovery, but after receiving a high-nitrogen solution for three weeks they began to show signs of wilting. The plants of the $-N_2$ group had large, dark green leaves on the upper part of the plant and small, pale green leaves on the lower part. Apparently, the application of nitrogen after 44 days of shortage benefited only the new growth. The plants of the $-N_3$ group showed slight improvement in size and color of the upper leaves (1).

The recovery of plants in the nitrogen groups, upon the addition of N after various periods of shortage, is given in centimeters of daily increase in height as follows:

After	27	days	of	shortage	1.080	cm.
"	44	"	"	"	1.007	"
"	58	"	"	"	0.700	"

The above data support the conclusion that the longer nitrogen is withheld, the slower is the recovery that the plant is able to make (5).

As stated before, the plants grown in a high-nitrogen nutrient solution tended to be 100 per cent. female while those deprived of nitrogen were 100 per cent. male. After an initial shortage of 27 days, plants of the $-N_1$ group were able to utilize the available nitrogen and the plants were all females. On the other hand, plants of the $-N_2$ group were apparently unable to use adequately the nitrogen which they received after 44 days of starvation, and male plants resulted. The plants of the $-N_3$ group had already begun to differentiate into males when the high-nitrogen solution was given to them after a shortage of 58 days. At the end of 64 days, plants of the $-N_4$ group were still hardy, growing slowly, and beginning to differentiate into male plants.

An examination of cross-sections of stems and leaves of hemp plants variously treated revealed certain significant differences. The stem tips of both

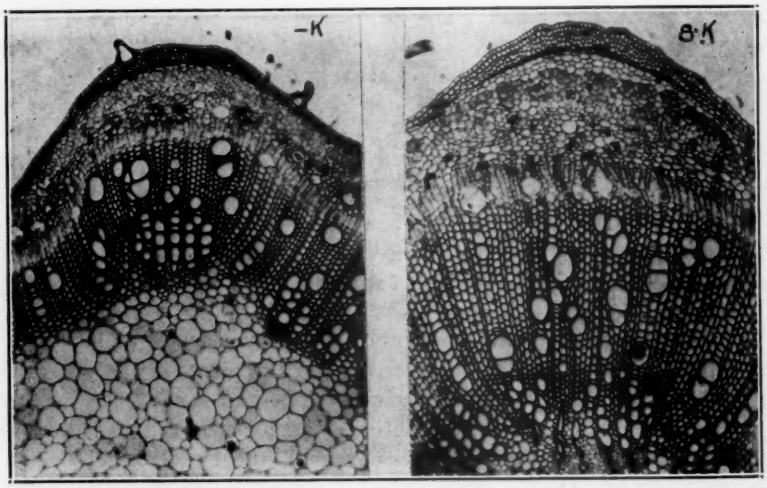

FIG. 9. Cross-sections of stem tips of 54-day-old hemp plants. Right, cross-section of stem of plant grown in high-potassium (8K) nutrient; left, cross-section of stem of plant grown in potassium-deficient (−K) nutrient.

the plants grown in the high-potassium (8K) and the potassium-deficient (−K) solutions (fig. 9) were fluted, and had mechanical tissue largely in the areas that bulged. Well defined cortex, phloem, cambium, and xylem were in evidence in both, although the stem of the plants grown in the high-potassium (8K) solution was much larger, measuring 1.9 mm. more in diameter at node 6 than that of the plants grown in the potassium-deficient solution (−K). Calcium oxalate crystals were numerous in the stem of the plants grown in the high-potassium (8K) solution but there were few of them in the stem of the plants grown in the potassium-deficient solution. Large inclusions, staining deeply red with haematoxylin, were found in the sieve tubes of both stems but they appeared more granular in the stem tip of the plants grown in the potassium-free (−K) solution. Inclusions of various sorts occurred in the cortex of both stems.

The stem tip of the plants grown in the high-magnesium (8Mg) solution had solid pith in contrast with the hollow pith of those grown in the magnesium-deficient (−Mg) solution, in which calcium oxalate crystals were more numerous. Large brownish inclusions, the resin of hemp, appeared in the phloem of the stem tip of plants grown in the high-magnesium solution and in the xylem of the stem tip of those grown in the magnesium-deficient solution.

The stem tip of the plants grown in the high-calcium (8Ca) solution showed numerous calcium oxalate crystals in the solid pith, which also contained a few calcium carbonate crystals. Mechanical tissue appeared largely in the areas of the stem that bulged. The bast fibers were better developed in the stem tip of plants grown in the high-calcium (8Ca) solution than in those grown in the calcium-deficient (−Ca) solution.

The stem tips of the plants grown both in the high-nitrogen (8N) and in the nitrogen-deficient (−N) solutions had a hollow pith; the former having some calcium oxalate crystals near the vascular tissue, and the latter considerable resin. More mechanical tissue had developed in the absence of nitrogen.

Examination of the vegetative stem tips of male and female plants failed to reveal any consistent outstanding variations, but the vegetative leaf of the female was slightly thicker than that of the male. The flowering stem tip of the male plant was 2.5 mm. in diameter and it had extensive xylem and little phloem. This contrasted with the female stem tip which was 5 mm. in diameter and had phloem as well as xylem well developed. Both had hollow pith with numerous calcium oxalate inclusions.

Summary

1. This investigation was concerned with the physiological effects of various nutrients on the hemp plant and the results of withholding single essential nutrients for varying intervals of time.

2. High-potassium (8K) nutrient (Knop's solution having 8 times the usual amount of potassium) produced the tallest and most vigorous plants which also had the largest and thickest leaves. Potassium-deficient nutrient (complete Knop's solution except for potassium) caused stunting of the growth of the plant and copper mottling on the leaves.

3. After variously prolonged periods of potassium starvation, hemp plants recovered rapidly but failed to attain a growth equal to that of plants which had a continuous supply of potassium.

4. Concentration of magnesium in the nutrient did not affect the growth of the plant, but magnesium deficiency resulted in chlorosis.

5. Recovery from magnesium starvation was slower the longer the magnesium was withheld.

6. Excess of calcium in the nutrient solution retarded growth. Calcium deficiency caused paleness in color, necrotic spots on the leaves, and early cessation of meristematic activity.

7. Recovery from calcium shortage in the hemp plant was more rapid after longer periods of starvation. Recovered plants attained greater height than those grown continuously in high-content solution.

8. High-nitrogen nutrient produced a dark green, leafy plant which did not survive. Plants grown in nitrogen-free solution were stunted and pale green.

9. After a short period of nitrogen starvation, hemp plants made a rapid recovery but died soon after. From longer periods of nitrogen shortage, recovery was slower.

10. An abundance of nitrogen at the time of fruit bud differentiation obviously leads to the production of female flowers, while the absence of nitrogen at that time tends to the production of male flowers.

Grateful acknowledgment is made to Dr. W. F. Loehwing for his direction and interest in this investigation.

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THE QUANTITATIVE MINERAL NUTRIENT REQUIREMENTS OF PLANTS

PAUL MACY

(WITH FIVE FIGURES)

Although the qualitative needs of plants for various mineral nutrients have been recognized for over a century, no method has been accepted for measuring the *quantitative* mineral nutrient requirements of plants. Herein is developed a relationship between the percentage content of a nutrient in a plant and the sufficiency of the nutrient for growth.

Review of literature and analysis of previous data

LIEBIG (**14**) considered the percentage nutrient content of plants as constant and the nutrient composition, therefore, as representing their nutrient needs. He proposed returning to the soil in the fertilizer all of the "ash" constituents of the crops removed. LAWES and GILBERT (**13**) found, however, that plants may need in the fertilizer more phosphorus than other nutrients although they contain less phosphorus than many other nutrients, so they concluded that the nutrient composition of the crop removed "is no *direct* guide whatever" to fertilizer needs.

WOLFF (**26**), using increments of nutrients in water cultures, reported the "minimum" requirements of the mature oat plant for the single nutrients in terms of their percentage content in the plant. His "minimum" percentage is really the *threshold optimum*, and it corresponds to the *critical percentage* as used herein. Although FRAPS, and PFEFFER have each reported this work by WOLFF, the method has not become accepted. WOLFF did not report the constancy of the nutrient requirements under various conditions, but his values of 1.0 per cent. N and 0.35 per cent. P_2O_5 for the oat plant agree well with the values obtained herein.

HALL (**10**) considered the idea of a critical proportion of each nutrient being needed in the ash of plants, but he concluded that, while the *supply* of a nutrient did affect its percentage content in the plant, the effect of other factors was as great or greater. This was the conclusion of almost all later investigators.

HALL's own paper contains the key to these erroneous conclusions. The phrase "will reflect the amount of plant food available in the soil, as measured by the *response* of the crop" embodies the concept of the *sufficiency* of a nutrient relative to all of the growth factors influencing yield rather than the absolute nutrient *supply*, about which HALL drew his conclusion. Table I presents the data selected by HALL to prove his point, together with

TABLE I

RELATION BETWEEN N CONTENT AND N RESPONSES OF WHEAT GRAIN
DATA FROM HALL AND FROM GILBERT

YEAR	PLOT 3 NO NITROGEN		PLOT 10 NITROGEN		RESPONSE
	YIELD	N	YIELD	N	
	bu.	%	*bu.*	%	*bu.*
1852 (poor)	13.9	2.08	21.9	2.48	8.0
1863 (good)	17.4	1.65	39.1	1.70	21.7

the yield and response data taken from GILBERT (9). (HALL's paper contains no response comparisons). While the data on nitrogen content show that season had more effect on the nitrogen content of the wheat than was caused by the nitrogen fertilization used, the *response* data show that when the percentage nitrogen content was high the nitrogen was more sufficient than when the nitrogen content was low. So it appears from these data that when other factors affect the percentage content of a nutrient in a plant they likewise affect the *sufficiency* of that nutrient as measured by *response* to it.

PFEIFFER *et al.* (21) proposed that there is a definite relationship between the percentage content of a nutrient in a plant and its sufficiency, as follows: "For those points on the yield curves at which the increase divided by the current yield (the sub-tangent) lead to the same value, the crops concerned show the same percentage nutrient content." They compared nutrient contents with yields rather than with yield responses, and they failed to designate the needed percentage of any nutrient. Their statement of the law is involved, and it may be stated more simply and more significantly as follows: *The sufficiency of a nutrient is a function of its percentage content in the plant.* The significance of this law was not realized by the writer until this work was completed.

They grew oats to the milk stage using different environmental series and increments of the nutrients concerned. In figure 1 is shown the relationship between the nitrogen content of the oats and the response to nitrogen under three different environmental conditions. (In the response comparisons herein, response is taken as the decrement from the maximum yield for that nutrient with other factors constant, and the approximate limits of error are indicated by double lines drawn by inspection.) The points in figure 1 fall on a narrow curve, which illustrates all of the concepts of the theory here proposed.

Outline of theory.—The central concept is a *critical percentage* of each nutrient in each kind of plant (1.0 per cent. N in this case), above which

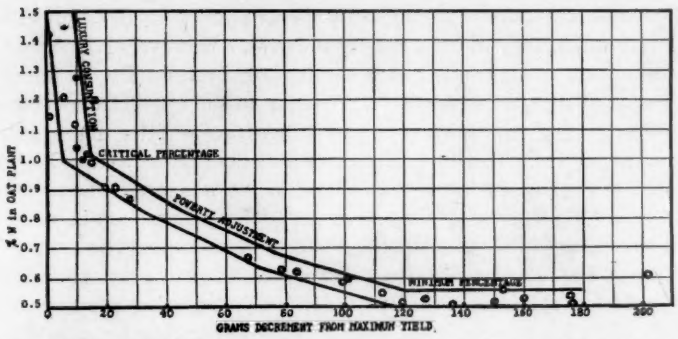

FIG. 1. The relation between percentage N content and N response of the oat plant at the milk stage. Data from PFEIFFER *et al.*

there is *luxury consumption* and below which there is *poverty adjustment,* which is almost proportional to the deficiency until a *minimum percentage* (0.5 per cent. N in this case) is reached.

In figure 2 A are shown yield curves from a similar experiment with phosphorus from PFEIFFER *et al.* (**21**). These distinct yield curves blend into a narrow phosphorus content-response curve in figure 2 B. The three points above the curve are from a series receiving aluminum, which may have entered the plant and fixed some of the phosphorus in an unassimilable form, as suggested by PIERRE and STUART (**22**). The laws of the minimum relationships shown in figure 2 A will be presented in the discussion.

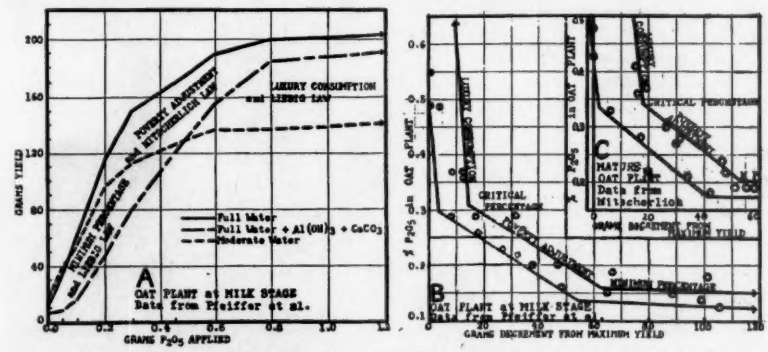

FIG. 2. A: Yield curves for oat plant with phosphorus showing relation between the Laws of the Minimum. B: Relation between percentage P_2O_5 content and P_2O_5 response of the oat plant at the milk stage. Same experiments as in A. Data from PFEIFFER *et al.* C: Same relation as in B with mature oat plants under other conditions. Data from MITSCHERLICH.

MITSCHERLICH (**16**) reported experiments similar to those of PFEIFFER *et al.*, with which they did not appear to agree. However, MITSCHERLICH'S data are shown in figure 2 C as a comparison between P_2O_5 content and P_2O_5 response, and the curve is very similar to the curve from PFEIFFER'S data. The critical and minimum percentages are a little higher, but the plants were mature. However, MITSCHERLICH (**17**) later criticized PFEIFFER very severely, and no further work along this line was reported by PFEIFFER.

MÜNTER (**18**) suggested limiting ratios between nutrients in wheat, but he concluded, as did HALL, that the effect of weather may be greater than the effect of fertilization. He reported the nutrient content and yields of wheat grain and straw from field plots for five years, using one increment of nutrient but with two sub-treatments. The comparison between the percentage nitrogen content of the grain from the no nitrogen plots and the response as shown by the nitrogen fertilized plots is shown in figure 3 A, and the same relationship for the straw is shown in figure 3 B. Aside from

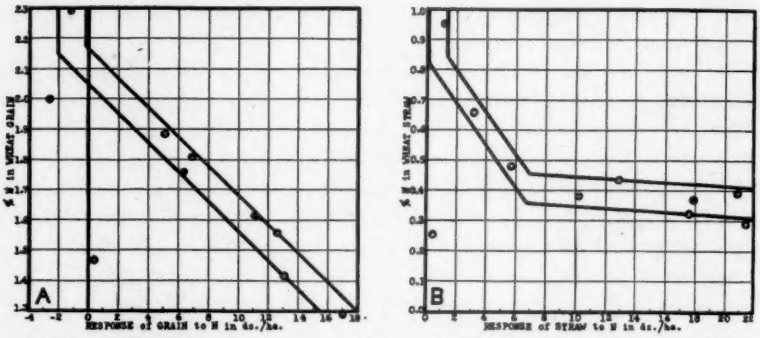

FIG. 3. Relation between percentage N content and N response of wheat grain (A), and straw (B). Data from MÜNTER.

one point (the same plot) in both cases (which may have been an error), the points fall on fairly narrow curves, particularly in the case of the straw, indicating a constant relationship between percentage nutrient content and nutrient sufficiency. MÜNTER'S data for phosphorus, however, showed considerable discrepancy, which may have been due to variable fixation of phosphorus in the plant as suggested above.

SALTER and AMES (**24**) considered the question of the use of plant composition as a guide to the availability of soil nutrients and they agreed with HALL'S conclusion. In figure 4 A is shown the comparison between the percentage potassium content of young corn on different plots and the response of the preceding five-year rotation to potassium, from the data reported by SALTER and AMES. The same relationship for alsike clover is shown in

figure 4 B. Again with one exception in each case, the points fit fairly narrow curves.

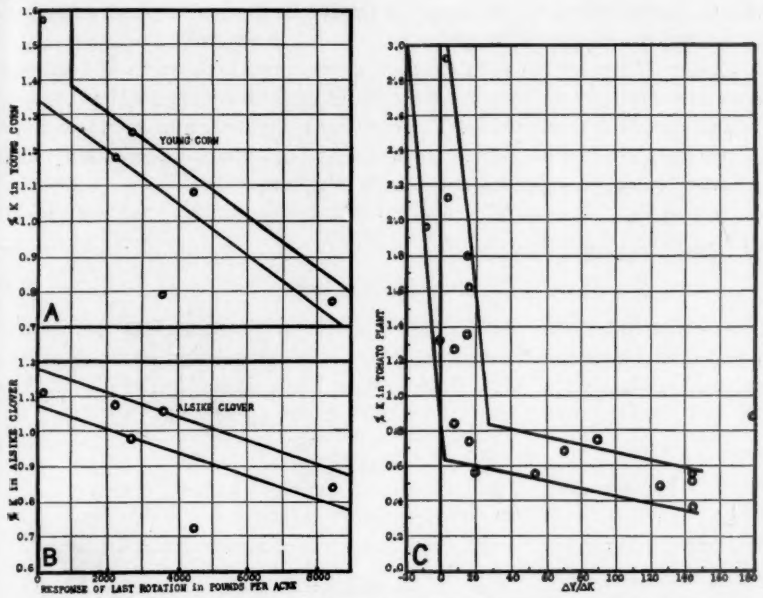

FIG. 4. A B: Relation between percentage K content and K response of young corn (A), and of alsike clover (B). Data from SALTER and AMES. C: Relation between percentage K content and K response of tomato plants at blossom stage. Data from HOAGLAND and MARTIN.

GERICKE (**8**) has recently suggested that there are definite minimum percentages of nutrients in plants, but he failed to report any.

HOAGLAND and MARTIN (**11**) reported some potassium nutrition experiments with tomato, which, with the unreported potassium contents obtained from Prof. D. R. HOAGLAND in a private communication, give a good comparison between the potassium content and potassium response of the tomato plant on 15 soils and during several seasons. Since the soils were reported to have high fixing capacities for potassium, the response has been calculated as the increase in yield per unit of increase in total potassium absorbed due to potassium fertilization, which is abbreviated to $\Delta Y/\Delta K$ in table II and figure 4 C. It is quite clear from figure 4 C that the tomato plant did not need over 0.9 per cent. K.

FRAPS (**5**) has reported the nitrogen content and the response to nitrogen of young corn grown on a large number of different soils in pots. If the percentage nitrogen content of the corn is compared with the response to

nitrogen, it is found that 82 per cent. of the crops having more than 0.70 per cent. nitrogen responded to nitrogen less than 10 grams per pot, while 82 per cent. of the crops having less than 0.70 per cent. nitrogen responded to nitrogen more than 10 grams per pot.

ALWAY (1) reported data showing a relationship between the sulphur content of alfalfa and response to sulphur, and he suggested the practical application of the relationship. The critical percentage appears to be about 0.2 per cent. S from his data. GARNER (6) has obtained preliminary data indicating the same value for sulphur in tobacco.

In 1934 GARNER *et al.* (7) reported a tentative critical percentage of 1.5 per cent. N in tobacco leaves.

Experimentation

In order to test further the theory of the critical nutrient composition of plants as a quantitative measure of the mineral nutrient needs of plants, it seemed advisable to study a single nutrient in a single kind of plant. The nitrogen requirement of barley was studied.

TABLE II

RELATION BETWEEN K CONTENT AND K RESPONSE OF TOMATO PLANTS
DATA FROM HOAGLAND AND MARTIN

SOIL NO.	CROP NO.	NO K POTS		K POTS		$\Delta Y/\Delta K$
		YIELD	K	YIELD	K	
			%		%	
29	2	86	2.92	90	4.40	3
21	2	82	2.12	86	3.32	4
101	2	103	1.96	91	3.72	−9
95	2	66	1.80	79	2.64	15
80	2	69	1.62	81	2.29	16
100	2	81	1.36	98	2.30	15
38	8	94	1.33	94	2.15	0
90	2	98	1.27	120	3.66	7
53	5	52	0.88	70	0.94	180
30	8	64	0.84	67	1.38	8
36	5	45	0.75	67	0.87	89
78	2	41	0.74	86	3.70	16
37	5	51	0.69	64	0.84	70
102	2	45	0.57	73	2.23	20
64	5	62	0.56	71	0.73	53
36	8	38	0.56	69	0.62	144
37	8	43	0.52	65	0.58	144
36	6	61	0.49	70	0.53	125
37	6	49	0.36	76	0.48	144

Alpha barley was grown on soil in gallon pots in triplicate in nine environmental series, each with no nitrogen and five or more increments of nitrogen applied as sodium nitrate. The symbols for the series treatments are defined as follows:

F means the first preliminary series,
V means Volusia silt loam soil,
D means Dunkirk fine sandy loam soil,
O means no fertilizer other than nitrogen,
P means 38 milligrams of phosphorus per pot,
K means 108 milligrams of potassium per pot,
L means 12 grams of limestone per pot.
25, 20, 15 indicate the percentage water content of the soil.
The Volusia is 25 when not indicated, and the Dunkirk is 12.

The preliminary series was planted on Dec. 3, 1933, and harvested on June 2, 1934, while the other series was planted on Feb. 14, 1934, and harvested on June 19, 1934. The vegetative response to nitrogen of the preliminary series was used as a basis for the nitrogen fertilization of the other series. This resulted in too much nitrogen to cover the grain curves very well. When harvested, the grain and straw were separated, weighed, and each analyzed for nitrogen by the Kjeldahl method (3). It was noted that the variation in the nitrogen contents of the replicates was relatively much less than the variation in the yields, the standard errors for the means of the triplicates being 0.22 gm. for the yields and 0.064 per cent. for the percentage nitrogen contents.

The yields and nitrogen contents of the grain are given in table III. The grain responses seem not to be reliable and are not given, because of the relatively large nitrogen increments and the resulting reductions in grain yields. These reductions are to a considerable extent complementary to increases in the straw yields, indicating the complicating phenomenon of competition between the grain and the straw for some other factor which becomes limiting. In table III the maximum yields are underlined and overscored, as is also the position which 2.2 per cent. N would occupy. This assumed critical percentage corresponds to an interval next to the maximum yield (where the maximum could just as well be) in all series except the V–PKL–25 series, of which the grain for the 158- and 237-milligram applications was badly smutted.

The data for the straw are given in table IV. The response is calculated as $\Delta Y/\Delta N$ for each increment and is compared with the average percentage nitrogen content for the corresponding interval. The yield curves for the straw shown in figure 5 A are well covered, and the series treatments had very marked effects on the yields. However, the relationship between the percentage nitrogen content of the straw and its response to nitrogen, shown

TABLE III

RELATION BETWEEN N CONTENT AND N RESPONSE OF BARLEY GRAIN

Mg. N applied	Yield	Percentage N	Yield	Percentage N	Yield	Percentage N
	D–O		V–O		V–PK	
		%		%		%
0	1.44	1.30	1.20	1.68	1.83	1.50
79	2.31	1.49	1.73	2.48	4.25	1.36
158	1.90	2.44	1.43	3.23	5.42	1.73
237	1.60	2.97	1.30	3.52	5.21	2.63
316	1.41	3.82	1.26	3.36	4.65	2.88
395	1.19	4.02	1.04	3.42	4.86	2.91
	V–PKL–25		V–PKL–20		V–PKL–15	
0	3.66	1.35	3.17	1.36	2.28	1.86
79	5.52	1.50	4.38	1.62	3.00	2.22
158	5.14	1.78	3.88	2.33	3.14	2.90
237	4.13	2.47	3.50	2.59	3.17	3.14
316	3.44	2.90	2.85	3.25	2.82	3.26
395	3.37	3.15	2.62	3.40	2.68	3.37
	D–PK		FD–PK		FV–PKL	
0	1.33	1.40	1.19	1.35	2.03	1.55
10			1.18	1.32		
20			1.22	1.54		
40			0.78	1.86	2.90	1.53
79	2.10	1.54	1.04	1.52	2.33	2.17
158	3.71	1.74			3.34	2.49
198			2.89	1.99		
237	2.08	2.31				
277					3.45	2.85
316	1.37	3.16				
395	1.25	3.80	2.11	3.81	3.06	3.06

in figure 5 B, is independent of these factors and is in agreement with the theory advanced. (Each of the three straight lines, in this case, has been fitted by the method of least squares. The broken lines represent the limits of two probable errors for a single point.) The critical percentage of about 0.8 per cent. N and the minimum percentage of about 0.4 per cent. N probably represent the immobile fraction of the nitrogen in the straw. These values are very similar to those for wheat straw in figure 3 B.

Discussion

PHYSIOLOGICAL SIGNIFICANCE

The critical nutrient composition of a plant is an inherent characteristic, which is probably seldom attained naturally. It might be called the "ideal"

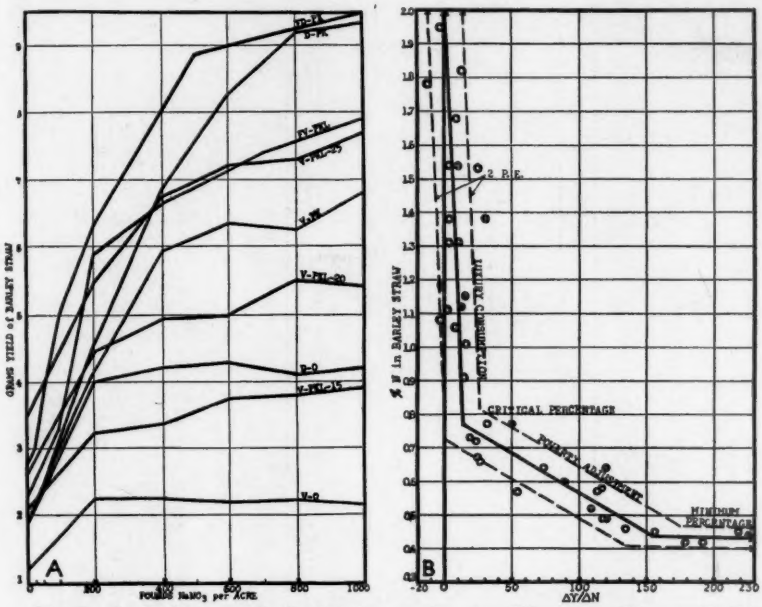

FIG. 5. A: Yield curves for barley straw under nine different conditions. B: Relation between percentage N content and N response of barley straw under the same nine conditions.

nutrient composition of the plant, because it represents a balance between the various nutrients and other growth factors, so that the nutrients are each just sufficient for the yield of the immediate plant. It represents a rational starting-point, at least, in the search for an "ideal" nutrient solution for the plant. Differences in the rates of absorption of the various nutrients may necessitate a modification of these tentative proportions. It is probable that such a solution, giving a nutritively balanced rate of supply to the plant, would be "physiologically balanced" also.

The form and distribution of a nutrient in the plant should be of value in discovering its functions, but only when the relative sufficiency of the nutrient is known, which can be evaluated in terms of the critical and minimum percentages of the nutrient in the plant.

While it is held that, when ordinary growth factors cause a variation in the percentage content of a nutrient in a plant, the percentage content of that nutrient which the plant needs is not changed thereby, it is not held that the critical and minimum percentages of nutrients in plants are absolutely invariable. The possible raising of the critical percentage of phosphorus by

TABLE IV

RELATION BETWEEN N CONTENT AND N RESPONSE OF BARLEY STRAW

Mg. N applied	Yield	Percentage N	Av.	ΔY/ΔN	Yield	Percentage N	Av.	ΔY/ΔN	Yield	Percentage N	Av.	ΔY/ΔN
	D–O				V–O				V–PK			
		%				%				%		
0	1.97	0.37			1.28	0.45			1.96	0.44		
			0.49	120			0.64	74			0.45	218
79	3.98	0.60			2.24	0.82			4.14	0.45		
			0.72	23			1.18	−1			0.52	108
158	4.23	0.83			2.23	1.54			5.97	0.59		
			1.31	3			1.95	−2			0.73	19
237	4.34	1.78			2.20	2.35			6.35	0.87		
			2.02	−14			2.59	4			1.08	−3
316	4.12	2.25			2.25	2.82			6.28	1.29		
			2.49	4			3.05	−24			1.38	30
395	4.19	2.72			2.07	3.27			6.82	1.46		
	V–PKL–25				V–PKL–20				V–PKL–15			
0	3.50	0.39			2.68	0.39			2.13	0.49		
			0.42	179			0.46	134			0.60	89
79	5.51	0.45			4.51	0.52			3.22	0.70		
			0.57	54			0.67	25			0.77	32
158	6.72	0.70			4.94	0.81			3.41	0.83		
			0.91	14			1.11	2			1.01	16
237	7.18	1.11			4.97	1.40			3.75	1.19		
			1.38	3			1.53	25			1.31	10
316	7.29	1.65			5.52	1.66			3.80	1.42		
			1.82	14			1.78	−12			1.54	10
395	7.70	1.98			5.40	1.89			3.91	1.65		

TABLE IV—(*Continued*)

Mg. N applied	Yield	Percentage N	Av.	ΔY/ΔN	Yield	Percentage N	Av.	ΔY/ΔN	Yield	Percentage N	Av.	ΔY/ΔN
	D–PK				FD–PK				FV–PKL			
		%				%				%		
0	1.97	0.37			2.75	0.45			2.30	0.39		
			0.45	156			0.45	309			0.39	280
10					3.02	0.44						
							0.44	226				
20					3.84	0.44						
							0.49	118				
40					4.99	0.53			3.28	0.38		
							0.57	113			0.42	192
79	4.55	0.52			6.34	0.61			5.88	0.45		
			0.58	116			0.64	120			0.66	26
158	6.84	0.64							6.71	0.87		
			0.77	50							1.12	12
198					8.82	0.67						
							1.06	8				
237	8.23	0.89										
			1.15	15								
277									7.38	1.37		
											1.68	9
316	9.22	1.40										
			1.54	4								
395	9.33	1.68			9.50	1.44			7.88	1.98		

aluminum has been mentioned; and there is, on the other hand, the possible lowering of the critical percentage of potassium by sodium. In fact, the answers to these unsettled questions lie in studies of these possible changes in the critical percentages. That is, by studies of critical percentages one may more definitely determine whether one element reduces the effectiveness of another, or may partly substitute for another.

The relationship between vegetation and reproduction in plants, which KRAUS and KRAYBILL (**12**) found associated with the carbohydrate-nitrogen ratio, is probably intimately connected with the critical percentages of the nutrients, particularly nitrogen, for these different types of tissues. Using the photoperiod as a variable, NIGHTINGALE (**20**) has confirmed KRAUS and KRAYBILL'S observations. It appears from NIGHTINGALE'S data that about 0.9 per cent. N in the dry weight of the stems of the tomato is necessary for blossoming and fruiting, and, while there may be blossoming at a much higher nitrogen content, there is no fruiting. It is probable that the form which the nitrogen takes in the plant is largely a function of its sufficiency, which can be measured in terms of the critical percentages.

RELATIONSHIP TO THE LAWS OF THE MINIMUM

LIEBIG (**14**) first proposed a Law of the Minimum, which states that plant growth is directly proportional to the supply of the nutrient which is in minimum. MITSCHERLICH (**15**) proposed a different law of the minimum, which states that the increase in yield per unit of limiting nutrient applied is directly proportional to the decrement from the maximum yield. MITSCHERLICH'S law has been very widely accepted, so much so that SPILLMAN (**25**) has gone so far as to hold that failure of data to agree with the law is in many cases due to large experimental errors in the data rather than to error in the law. However, RUSSELL (**23**) cites and supports much criticism of the MITSCHERLICH law.

In terms of the theory of the critical nutrient composition of plants both the LIEBIG and the MITSCHERLICH laws of the minimum hold over different portions of the yield curve, as is illustrated in figure 2 A. When the practically constant minimum percentage holds, the yield is directly proportional to the supply of the nutrient, which is the LIEBIG law. It will be remembered that LIEBIG had assumed a constant nutrient content in the plant. During poverty adjustment, however, the necessary percentage of the nutrient in the plant increases with the sufficiency of the nutrient, so that the response to the nutrient decreases progressively, which is the MITSCHERLICH law. When sufficiency is reached at the critical percentage, and luxury consumption sets in, the LIEBIG law again holds, there being no appreciable further response. So the LIEBIG law is the basic law, and the MITSCHERLICH law is a modification for the transitional phase. Although this phase is the part

of the yield curve most commonly met in practice, the MITSCHERLICH law should not be expected to fit the whole curve.

The principle of poverty adjustment corresponds to some other growth factor concepts. NICHOLS (**19**) has proposed a law of compensating factors, which states that the limiting effect of a factor may be compensated, within limits, by the relative favorable effects of other factors. Poverty adjustment is the mechanism by which this can occur with nutrients. In fact, the critical and minimum percentages set the limits within which it can occur. Poverty adjustment corresponds also to BENECKE's (**4**) concept of a factor being in "relative minimum," or only partially limiting.

That a nutrient may be only partially limiting is probably due to the almost *constant variation* of growth factors in nature, so that during the growth of a plant many factors may be limiting at different times. If this is true, it would be expected that, if all other factors were held absolutely constant, there would be no poverty adjustment and the critical percentage would be the same as the minimum percentage, which would be the only fundamental nutrient percentage. However, the widespread acceptance of the MITSCHERLICH law of the minimum, which is directly connected with poverty adjustment, indicates the constancy of the poverty adjustment relationship and, consequently, of the critical percentage.

AGRONOMIC SIGNIFICANCE

The most reliable method of determining the fertilizer needs of a crop on a soil is to make field plot tests. But even for this method to be accurate, increments of nutrients should be tested and many plots should be used over a field, both of which are seldom done. Because this method is inconvenient, and is becoming more so as more nutrients are found to need testing, many attempts have been made to measure the available nutrients in the soil by artificially imitating the action of the plant. Even the biological laboratory methods, and to a large extent the pot culture method, fail to take into consideration the differences in kinds of plants, the effect of climate on the plant and on the soil, and the physical condition of the soil in place. SALTER and AMES (**24**) have expressed the situation as follows: "All methods which are based upon a determination of the amount of a given nutrient present in the soil at a single sampling date and in such form as to be extractable under controlled laboratory conditions, whether they be physiological or chemical in nature, are subject to the same limitations in that they disregard the differential feeding power of plants and the dynamic nature of the plant-soil relationship." A chemical analysis of the particular crop grown on the particular soil under the particular local conditions to which the results will be applied has none of these limitations.

The practical value of this theory for the determination of the fertilizer needs of different soils is well illustrated by some data reported by AMES (2). The nitrogen, phosphorus, and potassium contents of wheat grown on two different experimental fields are compared in table V with the value of the

TABLE V

RELATION BETWEEN THE NUTRIENT CONTENTS OF WHEAT ON TWO SOILS AND THE NUTRIENT RESPONSES
DATA FROM AMES

FIELD	NITROGEN		PHOSPHORUS		POTASSIUM	
	N	VALUE OF INCREASE	P	VALUE OF INCREASE	K	VALUE OF INCREASE
	%		%		%	
Wooster	1.72	$10.19	0.334	$16.39	0.36	$4.58
Strongsville	2.09	1.05	0.327	21.79	0.41	0.90

crop increase during the last rotation due to fertilization with these nutrients. The nutrient contents of the wheat indicate that at Wooster, as compared with Strongsville, there is more deficiency of nitrogen and potassium and somewhat less deficiency of phosphorus. These indications are borne out very well indeed by the responses obtained.

Summary

A theory of the relationship between the sufficiency of a nutrient and its percentage content in the plant is proposed as a measure of the quantitative mineral nutrient requirements of plants. The central concept is a *critical percentage* of each nutrient in each kind of plant, above which there is *luxury consumption*, and below which there is *poverty adjustment*, which is almost proportional to the deficiency until a *minimum percentage* is reached.

The conclusions of previous investigators that other growth factors in general may also affect the percentage content of a nutrient in the plant have been met with proof from their own data, and from other data, that *the sufficiency of the nutrient is likewise affected* so that the above relationships still hold. The theory has been tested with nitrogen in barley under several conditions. These tests show that extreme effects of other factors on the yield and on the nitrogen content of the straw had no effect on the above relationships. The data for the grain indicate the same relationships but are uncertain because of smut and because of marked inhibiting effects of the high applications of nitrogen used.

It is proposed that the critical nutrient composition of a plant is an "ideal" but inherent characteristic of the plant, the critical and minimum

percentages varying only under special conditions. The critical percentages are believed to be intimately connected with the relationship between vegetation and reproduction.

It is shown that the MITSCHERLICH law of the minimum holds only during poverty adjustment, while the LIEBIG law of the minimum holds over the rest of the yield curve.

It is proposed that the use of the theory to determine fertilizer needs of particular crops on particular soils under the particular local conditions may meet the need for a convenient method as reliable as field plot tests.

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EFFECT OF BORON DEFICIENCY UPON THE STRUCTURE OF *ZEA MAYS*

ETHEL TABER ELTINGE

(WITH NINE FIGURES)

Introduction

Boron is now included, by most plant physiologists, among the elements essential for the normal growth of higher plants.

Until recently, most of the work has been done on the amount and habit of growth produced in many of our common plants, either by too great a quantity of boron or by a lack of it. BRENCHLEY (**1**) and COLLINGS (**5**) found boron to be toxic for both growth and germination with the concentrations which they used. Since then, by using very small amounts, BRENCHLEY and WARINGTON (**3**), and others (**9, 10, 12, 14, 15, 16, 18, 20, 21, 23, 24, 26, 27**), have found boron to be indispensable for the normal growth of the plants used.

Much less investigation has been done on the effect of boron on the internal structure of the plant. WEBBER (**31**) studied the effect of a toxic concentration of boron on prune, peach, and grape. She finds fewer chloroplasts in the leaves, and a disintegration of leaf tissue in the plants supplied with such a concentration. The smaller veinlets were often filled with a brown substance of gummy appearance. The petioles and midribs of prune often showed gum pockets resulting from a disintegration of the xylem elements. In shoots, the greatest injury was found in those that do not live beyond the first year. Sometimes phellogen developed beneath the necrotic tissues, often causing them to slough off. As in the leaf, gum pockets appeared both in the cortex and conducting system.

Several other investigators have shown the effect of a lack of boron on the structure of certain plants. WARINGTON (**28**) working with *Vicia faba* found that when boron was omitted from the nutrient solution, abnormal structure of the stem and the root resulted. Hypertrophy and disintegration of cambium in the stem tip was observed first and this was followed by disintegration of phloem and ground tissue. The xylem was often poorly developed, especially at the stem tip, the thin walled xylem elements being invariably absent. Disintegration of cells often resulted in blackened tissue where the cell wall was apparently primarily affected.

HAAS and KLOTZ (**13**) working with citrus have likewise found cambium and phloem disintegration followed by the formation of a copious amount of gum which reached the outside of the stem by splits in the cortex.

BRENCHLEY and THORNTON (**2**), in studying the effect of boron deficiency on nodule formation on *Vicia faba*, found some minute nodules buried in cortical tissue and having no vascular strands; other nodules were larger but with weakly developed vascular strands running a short distance into them. Plants supplied with boron, on the other hand, showed vascular strands extending the whole length of the nodule.

SOMMER and SOROKIN (**25**), in their work on the root tips of *Pisum sativum*, found that a lack of boron brought about an enlarged root tip showing no regularity of cell arrangement, and either no root cap or a deformed one. One of the most noticeable abnormalities was the appearance of the meristematic regions of lateral root primordia at a distance of not more than 10 to 13 cells from the root tip. These seldom developed enough to break through the epidermis.

MARTIN (**18**), in his experiments with sugar cane, found one of the first symptoms of boron deficiency to be a change in the lignified fiber cells on both the upper and lower sides of the chlorophyll bearing bundle sheath in the leaf. These appeared small, poorly developed, and loosely arranged. Some of the lower cells in the bundle sheath also often enlarged, even to the extent of pushing through the lower leaf surface, forming gall-like bodies between the large vascular bundles. Sections of young leaves showing deficiency symptoms, on the other hand, revealed deep constrictions in injured regions.

Materials and methods

Zea mays L. was chosen for this investigation because relatively little work has been done on the effect of boron deficiency on the structure of this plant or on other monocotyledons.

In order to obtain uniform material, the corn grains used were from the middle of a single ear of the horticultural variety white dent. The corn was obtained from Massachusetts State College. The grains were planted in flats of sand on June 12, 1934. At the end of 10 days, the seedlings were washed free from sand by tap water followed by distilled water. After being weighed and measured, they were supported by non-absorbent cotton in large, flat, paraffined corks which fitted quart mason jars. The jars were washed as free from boron as possible.

Shive's nutrient solution, with the addition of a small amount of manganese sulphate, was used for the plants lacking boron; for the control plants, a small amount of boric acid was added to the same solution. The salts used were of reagent grade and were not recrystallized. The water used in the experiment was distilled in a block tin lined copper still and stored in a similar tank until taken to the greenhouse in 5-gallon glass carboys. At no time

was the water allowed to stand in the carboys for more than a few hours. The solution was made up as follows:

KH_2PO_4 (M/2 solution)	36.00 cc.
$MgSO_4 \cdot 7H_2O$ (m/2 solution)	30.00 "
$Ca(NO_3)_2$ (M/2 solution)	10.00 "
$FeSO_4 \cdot 7H_2O$ (0.5 gm. per 100 cc.)	1.50 "
$MnSO_4 \cdot 4H_2O$ (0.18 gm. per 100 cc.)	0.56 "
H_3BO_3 (1 gm. per liter)	2.00 "

With the exception of the boric acid and manganese sulphate, these stock solutions were made up before each change of solution.

After the jars were filled with the nutrient solution, they were covered with brown paper. The solutions were changed twice each week, and each time the jars and the roots were thoroughly rinsed with fresh distilled water. Once a week the plants were measured and the leaves counted, and at the end of the experiment green and dry weights were made of all remaining plants.

This investigation was carried on by means of three series of cultures. Each series was composed of a group of plants grown in a nutrient solution which was deficient in boron, and a group of plants grown in a nutrient solution supplied with boron (the control group). In the culture designated as series I, 32 plants were grown for 2 weeks in full nutrient solution containing boron. At the end of that time 16 plants were thoroughly rinsed with distilled water and transferred to a nutrient solution lacking boron. The other 16 plants, used as controls, remained in the solution containing boron.

Series II consisted of 16 plants half of which, after being washed free from sand, were placed directly into a boron-deficient solution; the other half were placed in a boron-supplied solution.

Series III duplicated series II, except that the plants were younger at the time the experiment was started. This served a twofold purpose in that it showed the effect of boron deficiency upon plants of different age, and supplied plants for sampling before any visible injury appeared. It was soon realized that these samples would contain the most important evidence of the effects of boron deficiency upon the structure of the plant in question. As soon as plants in series I and II showed any visible change, samples of different tissues were taken at each change of solution. Samples of plants in series III were taken beginning with the fourth day, before any external change was evident, and thereafter at each change of solution. These were killed in chrom-acetic-osmic acid for 24 hours, washed in water, dehydrated, imbedded in paraffin, and stained in safranin and gentian violet.

Results

Plants in series I, which had had full nutrient solution containing boron for two weeks previous to being transferred to a solution that was deficient

in boron, averaged 6 leaves and 7 gm. in weight when transferred to the latter solution. The first visible injury from a lack of boron appeared on the seventh day in 50 per cent. of the plants used. At this time the older leaves showed yellow green between the veins, and the youngest visible leaf was darker green and did not unroll. Upon closer examination the youngest visible leaf was found to be stuck together by a colorless jelly in the position of the guttation drop. When this leaf was unrolled mechanically, colorless, parallel, transparent streaks were found running between the veins. The embryonic leaf within this was yellowish brown and appeared to be rotting.

At the end of 10 days, all of the plants having a deficiency of boron, in this series, showed similar injury. After a few days some of the plants showed the tightly rolled leaf torn apart by the protrusion of a leaf from within. After continuing to develop for a few days, the latter also showed boron-deficiency symptoms.

On the seventh day, the roots began to show injury. The tips showed enlargement and the growth of secondary roots became irregular. The abnormality in root growth increased so that at the end of three weeks the roots were brown in color and very brittle, tending to break at the slightest touch. They also showed secondary roots beginning to develop just back of the root cap. In some cases the root tip forked, forming branches of equal length. Figure 7 shows a typical plant (*A*) grown in a boron-supplied solution, and a plant (*B*) grown in a boron-deficient solution, at the end of 3 weeks growth.

Plants in series II, which were transferred directly from sand to a boron-deficient solution, began to show visible injury on the seventh day and continued to grow abnormally during the rest of the experiment. Figure 8 shows types of injury present after 10 days of growth in this boron-deficient solution. The plant on the extreme right shows a tightly rolled leaf being torn apart by the development of a leaf from within. Leaves formed after the plants were placed in the solution never showed well developed laminae. Often little more than a midrib developed, and this was usually much shorter than in a normal leaf. Sometimes several secondary branches developed, each showing deficiency symptoms after 4 or 5 days. Figure 9, *B*, shows such a plant, whereas plants *C* and *D* of the same figure show some abnormal leaves similar to those on the plants in figure 8.

The roots in series II showed the same abnormal development as those in series I, and they appeared at the same time.

Table I gives the increase in height and number of leaves, the green weight, dry weight, and percentage of dry weight after 3 weeks growth in nutrient solution having a deficiency of boron (−B), and supplied with boron (+B).

The plants grown in the boron-deficient solution (table I) contained a higher percentage of dry substance than did the plants grown in the boron-

TABLE I

EFFECT UPON PLANTS GROWN FOR 3 WEEKS IN NUTRIENT SOLUTIONS WITH AND WITHOUT BORON

	Increase in height		Leaves added		Green wt.		Dry wt.		Percentage dry wt.	
	−B	+B	−B	+B	−B	+B	−B	+B	−B	+B
	cm.	*cm.*			*gm.*	*gm.*	*gm.*	*gm.*	%	%
Series I	6.03	15.6	2.84	5.13	61.34	104.54	9.65	12.89	15.73	12.33
Series II	6.29	11.59	2.9	4.87	43.8	50.5	7.46	7.4	17.03	14.65

supplied solution. The data in table I also show that it makes little difference in the results obtained whether the plants are started in a boron-supplied solution and are then transferred to a boron-deficient solution, or whether they are grown from the beginning in a boron-deficient solution.

Studies of tissue from different parts of the individual plants in series III showed that injury appeared first at the end of 4 to 5 days in the young rolled leaves, while the plants were still normal in external appearance. Small scattered regions, in which the cells seemed to be collapsed and disintegrated, appeared in the innermost rolled leaf from near the base to above the middle (fig. 1). In addition, many of the air spaces seemed to be filled

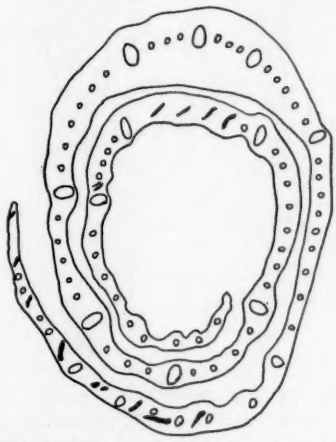


FIG. 1. Innermost rolled leaf in series III after growing in boron-deficient solution 5 days. Solid black areas show collapsed tissue.

with material which stained red with safranin. The colorless jelly which caused the leaves to stick together could be seen in many of the prepared

slides and seemed to resemble the material filling the air spaces. Parenchyma cells seemed to be the first cells affected. Soon there was also a change in the appearance of the cells in the small veins, which contained little if any lignified tissue. At the end of 10 days series III was discontinued because the plants in series II, after being in a boron-deficient solution for the same length of time, showed a comparable amount of injury both externally and internally. The only difference between series II and III was that the plants in series II were older at the time the experiment was started. Sections of plants in both series, at this time, showed that the two innermost leaves were affected; the outer leaf, which was more mature at the time of transfer to a boron-deficient solution, showed less injury than the inner leaf. The collapsed areas had enlarged and many parts of the laminae, with the exception of the midrib, had failed to differentiate into the usual tissues (fig. 2). The

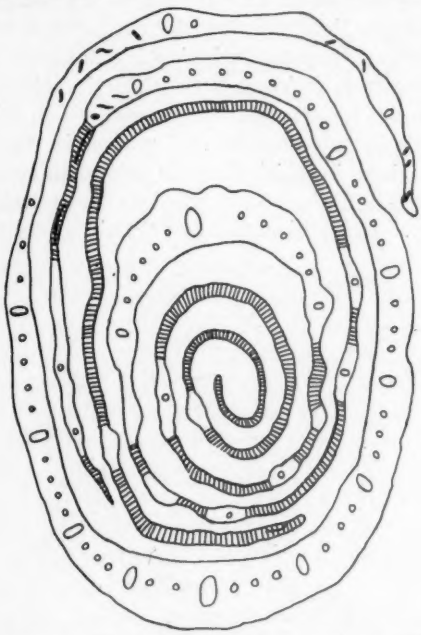


FIG. 2. Two innermost leaves in series II after growing in boron-deficient solution for 10 days. Cross-barred areas indicate undifferentiated tissue; solid black, collapsed tissue.

stem tip, however, was apparently normal. This stage in injury may be correlated with that present when the two inner leaves were stuck together. As injury progressed, the leaves, which had never unrolled, were often torn

apart (fig. 3, *A*) by the pushing through of leaves formed after the beginning of the experiment.

Leaves a little older than the two just mentioned, but still not mature when the experiment was started, often showed places in the cross-section where the entire leaf had collapsed, leaving only a thin band connecting such a region with the non-collapsed portion (fig. 3, *B*). Other places in the cross-

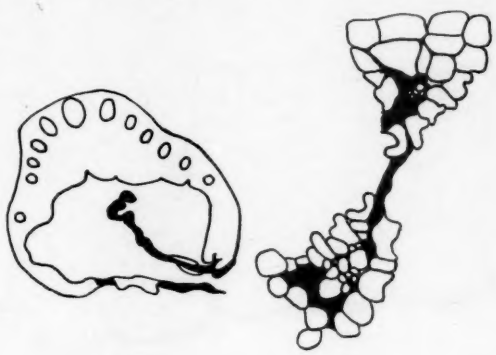


FIG. 3. *A*, injured leaf torn apart by leaves from within; *B*, partially mature leaf showing entire cross-section collapsed.

section showed hypertrophy of cells, especially those in the lower epidermis (fig. 4). Still other regions seemed normal, except for unusual thickness

FIG. 4. Partially mature leaf of plant, grown in boron-deficient solution, showing hypertrophy of lower epidermal cells.

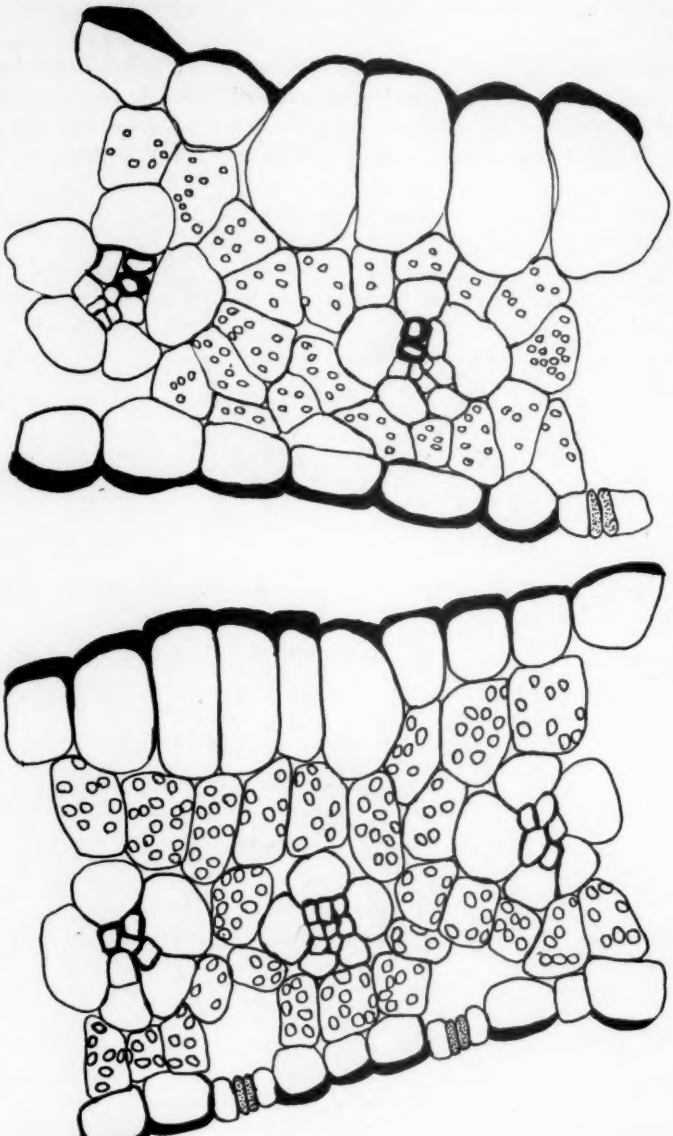


FIG. 5. *A*, (above) normal corn leaf; *B*, (below) leaf of same age, from plant grown in boron-deficient solution, showing injury by increase in thickness.

which seemed to be due to the presence of larger parenchyma cells, and sometimes to an additional layer of these cells (fig. 5, *A*, *B*). Leaves, mature at the time the experiment was started, showed very little injury. Those formed after the plants were put into a boron-deficient solution never had well developed laminae. Neither side of the lamina was as wide as in a normal leaf of the same age, and, in many cases, one side was scarcely developed (fig. 6).

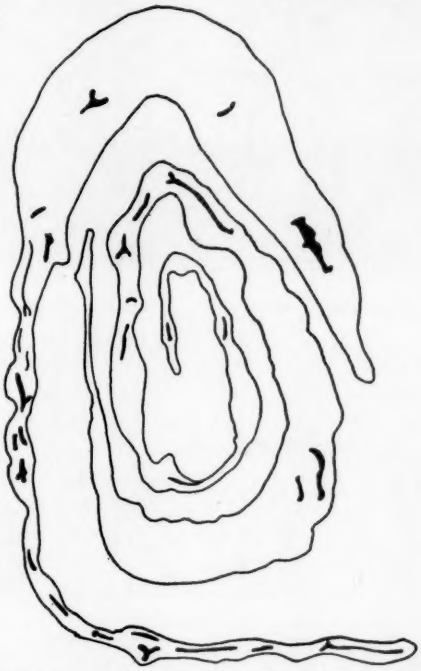
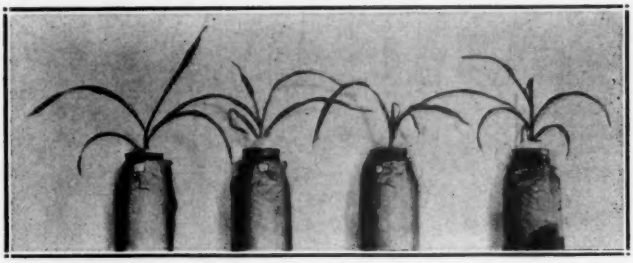
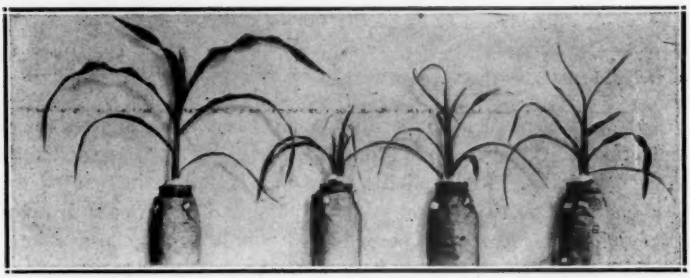


FIG. 6. Two leaves formed after plant was put in boron-deficient solution; showing poorly developed laminae.

Such leaves were also much thicker than normal leaves and showed almost no differentiation of tissues. Somewhat later these leaves showed disintegration in certain areas. At about the same time, small disintegrated regions appeared in the stem tip. Some plants at the end of 3 weeks growth in a boron-deficient solution showed secondary shoots beginning to develop. At such places large injured regions were often visible in cross-sections of the stem. After three and one-half weeks of growth in nutrient solution, the remaining plants were harvested because all plants deficient in boron had almost stopped growing, although the control plants were still growing vigorously.

A B





A B C D

FIGS. 7–9. **Fig. 7, series I: *A*, plant grown in boron-supplied solution 5 weeks; *B*, plant grown in boron-supplied solution 2 weeks, then grown in boron-deficient solution 3 weeks. Fig. 8, series II, plants grown in boron-deficient solution 10 days. Fig. 9, series II: *A*, control plant grown in boron-supplied solution 24 days; *B*, *C*, and *D*, plants grown in boron-deficient solution 24 days.**

Discussion

The results of this investigation show boron to be an essential element in the nutrition of *Zea mays*. Injury from boron deficiency can be seen in internal structure as early as 4 days after the plants have been placed in a boron-deficient nutrient solution, at which time the plants were still normal and healthy in external appearance. The lower portion of a partially mature leaf is the place where injury is seen first. By the time the plants showed visible external injury, marked internal injury had developed.

MARTIN (**18**), in his work with sugar cane, reported the enlargement of cells in the bundle sheath and also the enlargement of the fiber cells above and below the bundle. This did not occur in the corn leaves. Increase in size was found occasionally in some of the lower epidermal cells, and somewhat more frequently in the mesophyll cells. There was no formation of gall-like bodies on the under surface of the leaf as was recorded by MARTIN in sugar cane. The same constriction of leaf cross section that he reports for sugar cane is found in corn in the advanced stages of injury. The difference is that, in corn, the constriction appeared externally as a transparent streak and not a split in tissue; in sugar cane, the region very often split.

Gum and jelly formation so characteristic of corn plants grown in a boron-deficient solution has also been reported by HAAS and KLOTZ (**13**) in their work on deficiency symptoms in citrus, and by WEBBER, in prune and peach, when she used a toxic concentration of boron. Since this condition is present both with no boron and with a toxic concentration of boron, the condition can perhaps be interpreted as a physiological result of a disturbance in the growth mechanism owing to a large quantity of food material that has been carried to the meristematic region and cannot be used.

WARINGTON (**28**), in her work with *Vicia faba*, found the cambium and phloem cells of the stem tip among those first affected. In corn the mesophyll cells in the leaves seemed to be the first to show injury, and this is followed by injury of the tissue in the small veins. Injury in the stem tip was not visible until the end of the second week, and by this time advanced injury was present in the leaves. Leaves formed after the plants were placed in a boron-deficient solution seldom showed more than a few small regions where the cells differentiated and developed normally. Most cells, with the exception of those in the midrib, remained small and undifferentiated until they disintegrated.

Plants of *Zea mays* that were deficient in boron had a higher percentage of dry weight than did the control plants. This may be the result of the presence of a larger number of cells and of smaller cells in leaves formed after being placed in a boron-deficient solution.

A comparison of the age of the plants in series II and III shows that the age factor has little influence on the time required for injury to develop.

Also a comparison of the results obtained in series I and II show that previous treatment in regard to the presence or absence of boron had no effect upon the length of time necessary for the appearance of deficiency symptoms. These results indicate that the boron, which is absorbed from a nutrient solution, enters into combination with other elements, and it is used immediately by the plant; therefore, it is not available for translocation to other tissues or organs.

Summary

1. In cultures of *Zea mays* the first visible effect of a lack of boron appeared on the seventh day in the form of a chlorosis of the tissue between the veins of the older leaves, and the failure of the youngest leaves to unroll normally. A colorless jelly seemed to be the cause of the failure of the leaves to unroll.

2. Leaves formed after the beginning of the experiment were able, in some cases, to break through the leaves which were stuck together, and partially develop. These leaves never had well developed laminae, often no more than a midrib.

3. From the seventh day the root tips showed injury in the form of an enlarged tip, cheesy consistency, and the development of secondary root primordia very close to the root cap.

4. Plants that were deficient in boron had a higher percentage of dry weight than did the control plants.

5. The first internal deficiency symptom, seen in 4 to 5 days, appeared as a disintegration of parenchyma cells. This was accompanied by some of the air spaces becoming filled with material that stained the same as the jelly which caused the leaves to stick together.

6. A later stage in injury showed some parts where the entire cross-section of the leaf collapsed into a thin band, which stained red with safranin. Other places in the same leaf showed regions where the cells in the cross-section had failed to differentiate. Still other areas showed hypertrophy of cells, especially those in the lower epidermis. Some leaves were thicker than the control leaves. This seemed to be due to larger cells, and, in some cases, to an additional layer of cells.

7. At the end of two weeks growth in a boron-deficient solution, disintegrating cells were found in the stem tip. By the end of three weeks these areas had enlarged, being especially noticeable where secondary shoot primordia were developing.

8. The length of time required for the appearance of boron deficiency symptoms seemed to depend upon the length of time the plants had been in the boron-deficient solution, and not upon the age or previous treatment.

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TRANSPORT OF ROOT-FORMING HORMONE IN WOODY CUTTINGS

WILLIAM C. COOPER

(WITH EIGHT FIGURES)

It has been repeatedly observed since early times that if stems from some of our common woody plants are girdled, adventitious roots may be formed above the girdle. Although this regeneration phenomenon may result in part from an interruption of food transport, recent evidence indicates that the regeneration is controlled by hormones which are transported in the phloem. The greater part of the experimental work on the nature and transport of these hormones in the plant has been done with *Avena* coleoptiles and pea (*Pisum sativum*) cuttings. WENT (**12**) demonstrated that a growth-promoting factor from *Avena* coleoptile tips will diffuse out of them into a layer of agar, and that decapitated coleoptiles will grow considerably faster if blocks of this agar are placed on the cut surfaces than if they are supplied with pure agar. The chemistry of this growth substance has been worked out especially by KÖGL, HAAGEN SMIT, AND ERXLEBEN (**6, 7**). Three different crystalline substances were isolated, which physiologically cannot be distinguished. They have been named auxin a ($C_{18}H_{32}O_5$); auxin b ($C_{18}H_{30}O_4$); and hetero-auxin (indole-3-acetic acid—$C_{10}H_9O_2N$). Later THIMANN (**10, 11**) found that synthetic hetero-auxin not only promotes growth by cell elongation but also causes root formation on pea cuttings. COOPER (**2**) also has shown that hetero-auxin induces root formation on lemon, fig, *Acalypha,* and *Lantana* stem cuttings, and ZIMMERMAN and WILCOXON (**17**) have found that hetero-auxin causes local initiation of adventitious roots on stems and leaves of tomato and tobacco plants.

Experimental work, especially by VAN DER WEY (**15, 16**), on the transport of auxin in *Avena,* has shown that auxin is transported basipitally only, and that it is intimately connected with life processes. To cover these facts WENT (**13**) proposed the theory that naturally occurring potential differences in coleoptiles and other organs (apical parts being negative to the basal parts) would produce an electrophoresis of the negatively charged ions of auxin toward the positive base.

In addition, it was found by WENT (**14**) that the transport of substances causing root formation in *Acalypha* is rectilinear, rigorously polar, and independent of the transport of carbohydrates. In contrast to these results showing polar transport of hormones as a basis of the well-known morphological polarity in *Avena, Acalypha,* and other plants, HITCHCOCK and ZIMMERMAN (**5**) observed no strictly polar movement of several synthetic com-

pounds, including hetero-auxin, in either stems or leaves of the tomato and tobacco plants. They found that these substances, when applied to the soil in highly concentrated water solutions, were absorbed by the roots and were transported upward in the xylem in the transpiration stream. Also, that when water solutions were admitted through the cut surface of a stem or leaf there was a longitudinal movement in either direction through dead stem tissue, this movement being influenced by transpiration. Thus they concluded that living cells were not essential for the transport of these substances, and that the main channel of transport is in the transpiration stream.

These results obtained by HITCHCOCK and ZIMMERMAN, however, do not prove that the normal channel of transport of hormones in the plant is in the transpiration stream. The transpiration stream may possibly provide a means for the upward transport of substances absorbed from the soil by the roots, but it does not provide for the downward transport of hormones found in leaves (WENT, **14**, AVERY, **1**). It is hardly possible that they would normally move downward in the xylem in a direction opposite to the transpiration stream. That root-forming substances, extracted from leaves, do move downward in the stem has been shown by WENT (**14**) with *Acalypha* cuttings. COOPER (**2**) also has found that hetero-auxin, or some substance in the stem activated by hetero-auxin, will move downward in lemon cuttings. Therefore, with the above considerations in mind, it seems highly probable that root-forming substances normally move downward in the phloem. Results of girdling, and local chilling experiments presented in this paper, give evidence that root-forming hormones do move downward in the phloem in lemon and rose cuttings.

Synthetic hetero-auxin was used in all experiments and was applied to the cuttings either by the lanolin method of LAIBACH (**8**) or in water solution. In the former method, one part of hetero-auxin was mixed with 2000 parts of pure lanolin, *i.e.*, 0.5 mg. hetero-auxin per gm. lanolin.[1] A small portion of this paste, roughly about 10 mg., was smeared both on the cut surface of the apical end of the cutting, and on a small area of one side of the cutting near the top, which had previously been scraped to remove the epidermis and outer cortical layers. This paste was left on throughout the experiment. Water solutions of various concentrations were applied to either the apical or basal end of the cuttings by soaking 8 to 24 hours in the solution: after treatment the cuttings were placed in sand in the propagating frame. Control cuttings for the lanolin method were treated with pure lanolin, while control cuttings for the water-solution method were soaked in tap water for the same length of time and under the same atmospheric conditions as were the treated cuttings.

[1] This concentration was used in all experiments with the lanolin paste.

All lemon cuttings, usually about 12 cm. long and 5 mm. in diameter, were made from twigs of mature wood of the current season's growth, and were obtained in all instances from the same grove in Claremont, Calif.

The cuttings were set in sand either in a sash-covered propagating frame without bottom heat in the greenhouse or in a thermostatically controlled propagating frame at Torrey Pines, California.[2] At the latter place the temperature of the sand was kept at 85 to 88° F. At Pasadena the sand was usually held at a temperature near 75° F.

Girdling experiments

Three types of rings, as illustrated in figure 1, were used in these experiments. In type A (fig. 1) a complete ring of phloem about 12 mm. wide was removed from the stem of the cuttings about 4 cm. from the base. Type B was only partly ringed, a straight vertical bridge (a) about 2 mm. wide being left across the ring. Type C was only partly ringed, but in this case

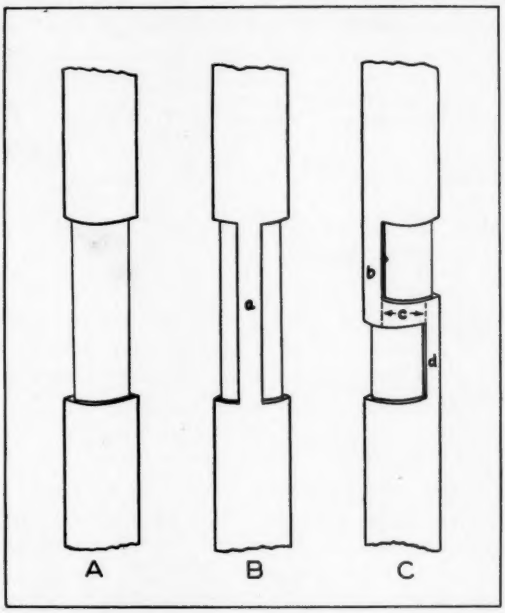


FIG. 1. Diagrams of different types of girdles used. A, complete ring of phloem removed. B, partly ringed with straight vertical bridge of phloem (a) left across the girdle. C, partly ringed with two-right-angled bridge of phloem (b, c, d) left across the girdle; (b) upper vertical arm, (c) horizontal arm, (d) lower vertical arm.

[2] The writer is indebted to Dr. L. C. MARSHALL of the U. S. Department of Agriculture Field Station, at Torrey Pines, California, for the use of the propagating facilities at Torrey Pines and for care of the cuttings while in the propagating frames.

a two-right-angled bridge of phloem (b, c, d) about 2 mm. wide was left across the ring. These methods of ringing are similar to those used by CZAPEK (**4**) in 1897 in studying the effectiveness of lateral transfer of food through stem cuttings of several woody plants.

Since these cuttings were kept in sash-covered propagating frames where the relative humidity was always high, it was not necessary to take the precaution of coating the exposed area with warm paraffin to prevent drying out of the xylem tissue, as was found necessary by CURTIS (**3**) with ringed tissue on trees out of doors. The fact that the cambium often became active and regenerated new tissue when the bark was removed is evidence that the xylem had not dried out.

The effect of ringing on root formation on lemon cuttings which had been treated with hetero-auxin is shown in table I and figure 2. When both

TABLE I

EFFECT OF RINGING ON ROOT FORMATION ON LEMON CUTTINGS TREATED WITH HETERO-AUXIN*

EXPT.	LEAVES	HETERO-AUXIN TREATMENT	WEEKS AFTER SET IN SAND	AVERAGE NUMBER OF ROOTS PER CUTTNG†		
				NOT RINGED	COMPLETE RING	NARROW BRIDGE OF PHLOEM STRAIGHT ACROSS RING
A	+	Lanolin mixture	6	4.8	0	3.4
		Pure lanolin	6	2.5	0	.....
B	–	0.5 mg./cc. water for 18 hours	3	4.2	0	1.1
		"	6	5.3	0.2‡	2.0
		Tap water 18 hours	3	0.2	0	0.3
		"	6	1.6	0	0.6
C	–	0.17 mg./cc. water for 15 hours	3	4.0	0	1.1
		Tap water 15 hours	3	1.7	0	0.0

* All cuttings treated at apical end.

† Average from 10 cuttings.

‡ Two roots on one cutting.

xylem and phloem above a complete ring were treated, either with hetero-auxin lanolin mixture, or with hetero-auxin water solution, roots formed at the base of the cuttings in only one out of 100 girdled cuttings; while similarly treated, non-ringed cuttings showed considerable root formation at the base of the cuttings. When a narrow vertical bridge of phloem was left across the ring, roots would form at the base, though in smaller number

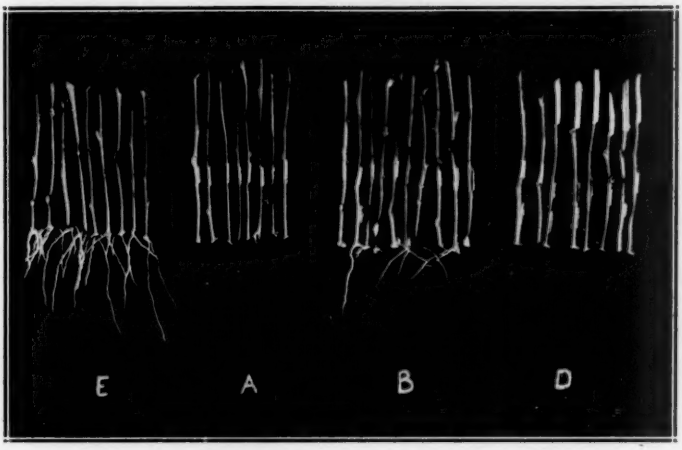


FIG. 2. Effect of ringing on root formation on leafless lemon cuttings treated at the apical end with a hetero-auxin solution (0.17 mg. per cc. of water) for 15 hours. E, not ringed; A, completely ringed; B, partly ringed with straight vertical bridge of phloem across the ring; D, same as B, except that one side of the apex of the cutting was removed in order that only the side of the apex opposite the phloem bridge was treated. Photograph taken three weeks after treatment.

than on the non-ringed cuttings. This is very good evidence that the hetero-auxin, or some root forming substance present in the cutting and activated by the hetero-auxin, is transported downward mainly in the phloem.

Root formation at the base of the cuttings with a two-right-angled bridge of phloem across the ring (fig. 1, C) was found to be influenced by the horizontal distance (c) between the side of the upper vertical arm (b) and that of the lower arm (d). When this distance was greater than 2 mm. there was usually a strong callus development at the sides and base of the upper vertical arm, but very little on the horizontal arm, and none on the lower vertical arm. In some instances roots would appear at the base of the upper vertical arm, but none on the horizontal arm nor at the base of the cutting. However, in cuttings where the horizontal arm (c) was less than 2 mm. long, an occasional root would form at the base of the cuttings; but at no time was root formation as great as on cuttings with a straight vertical bridge across the girdle.

In another experiment with two lots of partly girdled cuttings (type B), hormone solution was applied in one lot to the entire cut end of the apex (fig. 2, B), and in the other lot only to the side of the apex opposite the phloem bridge across the ring (fig. 2, D). No roots were formed on the

cuttings to which hormone was applied only on the side opposite the bridge; but in the other lot, roots formed at the base of the cuttings. These results, along with those obtained with the two-right-angled type girdle, indicate clearly that the hormone moves downward in the phloem, mainly in straight lines parallel to the phloem elements.

MacDaniels and Curtis (9) report that when lateral transfer of food in apple tree trunks was forced by spiral ringing, more rapid lateral conduction was provided for by structural changes in the phloem, beginning soon after ringing and resulting in the re-orientation of the cambium so as to be parallel with the spiral ring. They state that partial accommodation to the changed condition of conduction probably occurred immediately after ringing, by the first formed elements being connected through their radial walls. Perhaps this would account for the slight lateral movement of the root-forming hormone observed with the two-right-angled type of girdle on lemon cuttings.

Local chilling experiment

Chilling at 33 to 40° F. and at 38 to 46° F. of about 50 mm. of the stems near the mid-portion of leafless lemon cuttings was accomplished by inserting the cuttings through special insulated chilling units. These were set up in the propagating frame so that bases of the cuttings were in the sand at a temperature near 80° F., the middle of the cutting in the chilling device, and the tips, treated with hetero-auxin-lanolin paste, in the air at a temperature near 70° F. The chilling unit consisted of small water-tight tin cans into which were soldered 10 copper tubes in a vertical position and parallel to each other. This arrangement of copper tubes permitted insertion of cuttings through the device so that when cold water was circulated through the can, the portions of the cuttings in the tubes were chilled without actually coming in contact with the water. Two such chilling units, insulated with one-half inch of celotex, were connected with rubber tubing. Water at about 32° F. was circulated at a slow rate through the two-unit system for two weeks. Cuttings in the first unit were chilled locally to 33 to 40° F., and in the second unit to 38 to 46° F.

After two weeks of chilling, the hetero-auxin-lanolin paste was removed from the tips, the cuttings were taken out of the device and reset in the sand. There was no sign of callus formation at the base of either of the chilled lots, while a strong callus had formed on the cuttings which had been treated with hormone but not locally chilled. One week later, or three weeks from time of setting in sand, roots appeared on the non-chilled cuttings, while roots did not appear on the chilled cuttings until three weeks after chilling, or five weeks after setting in sand, and then largely on the 38 to 46° lot (table II). Thus we see that root formation on the

TABLE II

INFLUENCE OF LOCAL CHILLING OF LEMON CUTTINGS ON DOWNWARD MOVEMENT OF ROOT-FORMING HORMONE*

HETERO-AUXIN	CHILLING TREATMENT	AVERAGE NUMBER OF ROOTS PER CUTTING			
		END OF 2 WEEKS CHILLING	ONE WEEK AFTER CHILLING	THREE WEEKS AFTER CHILLING	FIVE WEEKS AFTER CHILLING
–	Not chilled	0	0.4	.....	1.8
+	" "	0	0.7	2.4	2.8
+	Chilled 33–40° F.	0	0.0	0.1	0.4
+	Chilled 38–46° F.	0	0.0	0.7	0.9

* Hetero-auxin-lanolin paste applied at top of cutting above chilled area.

locally chilled cuttings was delayed by two weeks—the length of the chilling period—and that root formation on chilled cuttings never did approach that of the non-chilled lot. Local chilling, then, must have acted to prevent the transport of hetro-auxin downward, because the few roots which formed during the five weeks after chilling can be accounted for by naturally occurring root-forming hormones in the stems. This seems obvious, since the untreated cuttings showed more roots than were formed on the chilled cuttings.

This evidence that local chilling actually does prevent the downward transport of hetero-auxin indicates that living cells take an active part in the transport of hetero-auxin or of some substance in the stem activated by hetero-auxin. It therefore seems well established from the combined results of the girdling and local chilling experiments that the hormone is transported downward in the phloem.

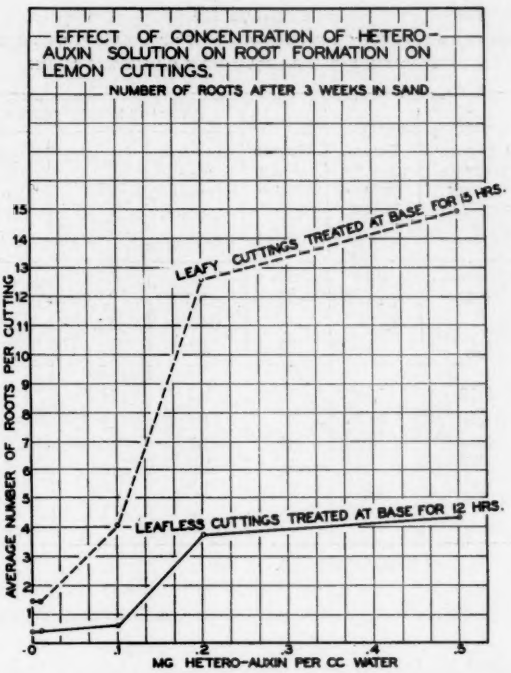
Effect of basal application of hetero-auxin solution on root formation

By soaking the base of lemon or rose cuttings in hetero-auxin solution of relatively high concentration (0.5 mg. per cc. of water) for eight hours before setting in sand, a much greater number of roots was induced on the treated than on control cuttings (figs. 3 and 4). Also it was found that the number of roots were from two to five times as great when treated at the base as when treated at the tip, provided a highly concentrated solution was used. The definite relation between the time of treatment, concentration of solution, and number of roots formed has not been determined. However, results shown in figure 5 for basal treatment of 0.04, 0.1, 0.2, 0.5 mg. hetero-auxin per cc. of water indicate that basal treatments are effective

FIG. 3. Lemon cuttings treated at the base with hetero-auxin solution (0.5 mg. per cc. of water) for 8 hours. Photograph taken 2½ weeks after treatment.

only in concentrations of 0.1 and above, for a 16-hour treatment of leafy cuttings, and 0.2 and above, for a 12-hour treatment of leafless cuttings. More dilute solutions applied at the base caused no more root formation

FIG. 4. Lemon cuttings treated at the base with tap water for 8 hours. Photograph taken after 2½ weeks.


EFFECT OF CONCENTRATION OF HETERO-AUXIN SOLUTION ON ROOT FORMATION ON LEMON CUTTINGS.
NUMBER OF ROOTS AFTER 3 WEEKS IN SAND
LEAFY CUTTINGS TREATED AT BASE FOR 15 HRS.
LEAFLESS CUTTINGS TREATED AT BASE FOR 12 HRS.
AVERAGE NUMBER OF ROOTS PER CUTTING
MG HETERO-AUXIN PER CC WATER

than tap water. It was noted that 0.5 mg. per cc. solution applied at the base caused some injury; so very likely the effective range of concentration for basal treatment of lemon cuttings for 12 to 15 hours is from 0.1 or 0.2 to near 0.5 mg. per cc.

Some idea as to the reason for this rooting response to basal treatments of hetero-auxin solution is obtained from a study of table III. Cuttings in experiments A and B were placed in a nearly saturated atmosphere in the propagating frame at Torrey Pines, during the period when the bases of the cuttings were soaked in hetero-auxin solution; thus very little opportunity was afforded for transpiration. Yet, in both instances, there was considerable increase in number of roots induced on the treated cuttings over that of the controls. However, when the portion of the base of the cutting which was actually in the hetero-auxin solution (about three-fourths of an inch) was cut off, the effect of the treatment was eliminated. This suggested that there was very little or no movement of the hetero-auxin solution up the stem. The solution was effective, however, only when applied at the cut end; for when the cut end was sealed over with paraffin before soaking in the hetero-auxin solution, and the paraffin later removed

TABLE III

EXPERIMENTS SHOWING THE EFFECT ON ROOT-FORMATION OF TREATING THE BASAL ENDS OF LEMON CUTTINGS WITH WATER SOLUTIONS OF HETERO-AUXIN

EXPT.	LEAVES	CONC. OF HETERO-AUXIN SOLUTION	DURATION OF TREATMENT	WEEKS AFTER TREATMENT	AVERAGE NUMBER OF ROOTS PER CUTTING*			
					TAP WATER CONTROL	BASE TREATED	BASE TREATED AND PART TREATED CUT OFF	BASE TREATED, CUT OFF, AND NEW BASE TREATED
		mg. per cc.	*hr.*	*wk.*				
A ...	+	0.5	20	2½	1.5	20.4	2.4	.....
				6	4.3	33.3	3.6	.....
B ...	+	0.1	8	2½	1.1	4.0	2.5	.....
				6	4.0	8.3	4.3	.....
C ...	+	0.17	15	3	1.7	12.6	4.9	4.8
	−	0.17	15	3	1.7	4.0	1.2	2.3
D ...	−	0.5	12	3½	1.1	4.3	0.9	0.8
				5½	1.8	5.9	1.7	1.6
	−	0.1	12	3½	1.1	0.6	0.8	0.5
				5½	1.8	1.6	1.6	1.4

* Average of 10 cuttings in each instance.

after the treatment, there was no increase in number of roots over the controls.

In experiment C, leafy lemon cuttings were exposed to laboratory air (temp. 70° F., relative humidity about 75 per cent.) during the 15 hours that the cut end of the bases were in hetero-auxin solution, thus affording opportunity for transpiration. As a consequence, an average of 2 cc. of solution was taken up by each cutting, probably in the xylem in the transpiration stream. As a result of these conditions, the number of roots on the cuttings with the treated portion of bases cut off was greater than the controls, yet the number was only about one-third that of the cuttings from which the treated portion of the base had not been cut off. This probable upward movement of the hetero-auxin in the xylem should not be mistaken, however, for the normal movement of naturally occurring hormones in the plant, because, as stated by CURTIS (3) "When solutions of most any sort of substance are introduced into cut stems these solutions are carried extensively and almost exclusively in the xylem tissues and it is obvious that transpiration determines both the direction and rate of movement of the solution introduced."

In experiment C, evidence of possible upward movement of hetero-auxin in the transpiration stream is of little significance; but the fact of greatest interest is that, if the treated portion at the base of a cutting was excised

and the base again treated, the number of roots formed was no greater than that of a similar cutting which had not been re-treated. This was observed again with leafless lemon cuttings in experiment D, in which a solution of 0.5 mg. of hetero-auxin per cc. of water was used. In this instance the number of roots on cuttings with treated base cut off, and on similar cuttings with base treated again, was the same as on controls; while treated cuttings, but with the base not removed, showed nearly three times as many roots as the controls. Results similar to these were also obtained with leafy cuttings of the rose (var. Lady Perkins).

It is clear from these results that there is some factor other than hetero-auxin concerned in root formation. If only hetero-auxin were involved, re-treating the cutting after cutting off the treated base should give nearly the same response as the original treatment. In order to insure equal amounts of stored food and other substances, the final length of the cuttings in all experiments was made the same. Cuttings whose treated bases were to be cut off were made longer in the beginning, to allow for the removal of a portion of the stem at the base.

Summarizing, we find that (1) when applied at the base of cuttings for 15 hours, only a strong solution of hetero-auxin is effective in inducing root formation; (2) cutting off the treated portion of the base nearly eliminates the effect of the treatment; (3) re-treating after cutting off the treated base causes no more roots than when not re-treated. To explain these facts, it is suggested that a strong solution of hetero-auxin, when applied to the base, causes the rapid movement downward of a substance, rhizocaline, present in the leaves and stem, which is necessary for root formation. Cutting off three-fourths of an inch of the base after treatment, cuts off most of the supply of rhizocaline and further treatment with hetero-auxin has little effect since hetero-auxin is only one of the substances necessary for root formation.

The effectiveness of treating the basal ends of lemon or rose cuttings with a strong solution of hetero-auxin in inducing root formation suggests a new line of attack in efforts to root cuttings of apple and other woody plants which do not root readily from cuttings. With lemon, rose, holly, and *Chrysanthemum* cuttings this method of treatment was much more effective than the lanolin method. The maximum number of roots per cutting obtained on lemon cuttings by the lanolin method was eight, while as many as seventy-five were obtained by soaking the base in hetero-auxin solution (0.5 mg. per cc. of water) for eight hours. The same concentration of solution induced the formation of twenty-five roots per cutting on the Cecil Bruner rose, and fourteen roots per cutting on a species of Chinese holly (*Ilex cornuta*), while the lanolin method produced only five roots per cutting on the rose, and none on the holly. Even more striking results were

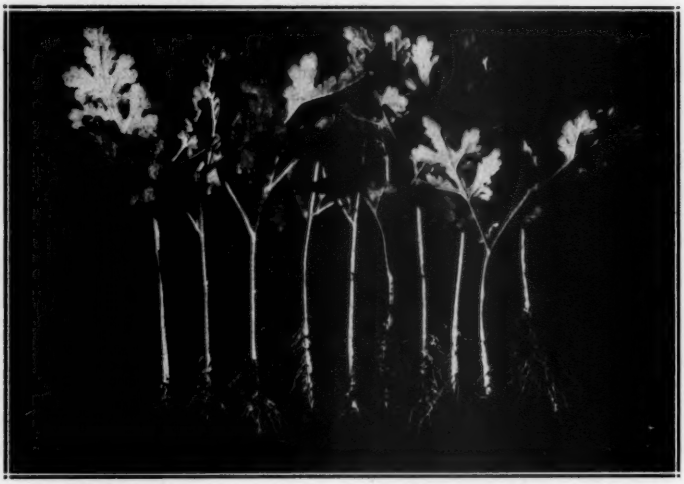


FIG. 6. *Chrysanthemum* cuttings treated at the base with hetero-auxin solution (0.1 mg. per cc. of water) for 15 hours. Photograph taken 2 weeks after treatment.

FIG. 7. *Chrysanthemum* cuttings treated near the top by the lanolin method. Photograph taken 2 weeks after application of hetero-auxin lanolin mixture.

FIG. 8. *Chrysanthemum* cuttings treated at the base with tap water for 15 hours. Photograph taken 2 weeks after treatment.

obtained with *Chrysanthemum* cuttings as is shown in figures 6, 7, and 8; but in this instance a hetero-auxin solution of 0.1 mg. per cc. of water was found most effective, as stronger solutions were toxic to the tissues of the base.[3]

Summary

1. Ringing experiments with lemon cuttings indicate that hetero-auxin, or some substance in the stem activated by hetero-auxin, is transported downward in the phloem and in a straight line parallel to the phloem elements.

[3] Since preparing this paper it has come to the attention of the writer that A. E. HITCHCOCK and P. W. ZIMMERMAN (Contrib. Boyce Thompson Inst. 8: 63–79. 1936) obtained excellent results with the rooting of certain species of *Ilex* and *Taxus* by soaking the bases of cuttings in water solutions of hetero-auxin in a manner similar to the method described in the present paper. These authors in discussing a former paper by the present writer, published in PLANT PHYSIOLOGY (2) state "The qualitative and quantitative differences resulting from treatment of lemon cuttings having leaves are not clear, since there is no illustration of control lots with which to compare the three treated lots shown." Reference to the article will show that in table I, page 790, are given the number of roots per cutting formed after two, three, and four weeks, on both treated and untreated leafy lemon cuttings. Although these figures in themselves provide complete proof of "qualitative and quantitative differences," there are also included illustrations of both treated and control cuttings. Reliable data on rooting can not, of course, be derived from illustrations alone; therefore in this paper both photographs and tables are included.

2. Local chilling experiments indicate that living cells take an active part in the downward transport of the hormone and supports the evidence, obtained in the ringing experiments, that downward transport of the hormone takes place in the phloem.

3. When the cut end of the base of a leafy lemon or rose cutting is placed in hetero-auxin solution there is some upward movement of the solution depending on the rate of transpiration.

4. When solutions of hetero-auxin are applied at the base of cuttings for 15 hours, only strong ones are effective in inducing root formation.

5. Cutting off the treated portion of the base of cuttings nearly eliminates the effect of the treatment. This is especially true in cases where treatments were made in a saturated atmosphere.

6. Re-treating with hetero-auxin solution after cutting off the treated base of cuttings causes no more roots than when not re-treated.

7. The hypothesis that a strong solution of hetero-auxin, when applied to the base of a cutting, causes the rapid downward movement of a substance, rhizocaline, which occurs in the leaves and stem, and which is necessary for root formation, is offered as an explanation of the facts stated in 4, 5, and 6.

8. The effectiveness of treating the base of cuttings of lemon or rose with strong solutions of hetero-auxin to induce root formation suggests a new line of attack in efforts to root cuttings of apple and other woody plants which do not root readily from cuttings.

These investigations were conducted at the William G. Kerckhoff laboratories of biology, California Institute of Technology. Grateful acknowledgment is made to Professor F. W. WENT for helpful suggestions and guidance during the course of the experiments and during the preparation of the manuscript.

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NUTRITIONAL REQUIREMENTS OF THE PATHOGENIC MOLD *TRICHOPHYTON INTERDIGITALE*

WILLIAM A. MOSHER, DONALD H. SAUNDERS, LYLE B. KINGERY, AND ROGER J. WILLIAMS

Introduction

Although, since the classical work of SABOURAUD, a number of investigations have been conducted dealing with the culturing of the *Trichophyta* and other pathogenic fungi (**2, 3**), very little information of a definite chemical nature is available regarding the nutritional requirements of these organisms. For this reason the writers have made an exhaustive study of these requirements in *Trichophyton interdigitale*. Peptone, or some such material of indefinite and unknown composition, has always played an important rôle in the nutrient medium used.

Lack of critical information and possible usefulness in the study of disease-producing activities prompted this investigation. The specificity of this organism and others for certain types of skin or for certain regions on the body is often striking and the writers assume that it must have some nutritional basis.

Experimentation

In this investigation a study has been made of the following requirements for *Trichophyton interdigitale:* (1) mineral salts, including the "trace" elements; (2) carbohydrates of various sorts; (3) specific amino acids and combinations thereof; and (4) specific nutrilites or vitamin-like substances. These requirements were not studied in the order indicated.

For this study, it is apparent that all indiscriminate mixtures such as peptone, yeast extract, meat extract, and others must be avoided, and only pure chemical substances used. The two exceptions to this were the use of pantothenic acid (**6**) and crude lactoflavin (**4**). Of these the former was highly concentrated and probably biologically pure; the latter was also highly concentrated so that in order to be effective less than 1 γ (0.000001 gm.) per cubic centimeter of culture medium was used. The other nutrilites used in pure form were crystalline vitamin B_1 and inositol, both of which have been shown to be effective in the nutrition of certain yeasts (**8**), and pure lactoflavin which is effective in animal nutrition (**1**).

In all tests on *Trichophyton interdigitale* the following method was employed in culturing, handling, and recording the growth under various conditions:

All cultures were grown in thin-walled Erlenmeyer flasks of 100-ml. capacity which had been thoroughly cleaned with hot chromic-sulphuric

acid cleaning solution. The nutrient solution of each flask was made up to 12 ml. with distilled water, and the mouth of the flask was plugged with autoclaved cotton. Each flask and its contents were then sterilized for 10 minutes in an Arnold steam sterilizer. More severe heat treatment was avoided because of decomposition (particularly of the nutrilites) which might result. Before seeding with the organism, the flasks were allowed to cool to room temperature because the spores of *T. interdigitale* are killed at 56° C. in 10 minutes. Temperatures lower than 56° C. decrease the vitality of this organism.

The stock cultures from which the seedings were made were transferred every month and were then allowed to grow for about two weeks before being used again. The cultures, whether on agar slants or in liquid media, seemed to grow best when the pH was adjusted to 5.5. A quinhydrone electrode was used for the pH determinations and sulphuric acid and sodium hydroxide were used in making the pH adjustments. That there was always a production of base by this organism was evidenced by the fact that after 20 days of growth in SABOURAUD'S medium the pH rose to 8. This is a striking rise when one considers that peptone and amino acids are effective buffering agents.

The seedings were made by adding a loopful of mycelium and spores from a mature culture of *Trichophyton interdigitale* grown on a slant composed of SABOURAUD'S dextrose medium to 10 ml. of sterile water. This was shaken vigorously and the resulting suspension observed under the low power of a microscope in a Levy counting chamber. If the number of spores in the total ruled field (0.0009 ml.) was greater than 30 the suspension was diluted 200 times; if 30 or fewer it was diluted 100 times. To each sterilized flask was added 0.1 cc. of the diluted spore suspension corresponding to approximately 30 spores per culture. The purpose of using very dilute suspensions was to avoid the contaminating influence of stored material which is introduced when the seedings are large. The seeded flasks were placed in a closed room where there were almost no temperature changes. Better and quicker growth was observed when the cultures were not placed in a 37° C. incubator.

The method of recording the growth was a modification of that described by WILLIAMS and HONN (**6**). As soon as growth was evident the cultures were carefully examined and compared. The culture showing the best growth was used as a standard culture. This standard culture was given the arbitrary rating of 10. The next best culture was given a rating in comparison with the standard. If the difference in growth was small the next flask in value was given the comparative rating of 9.5; if the growth was only half as much the rating was 5; if there was no growth the rating was 0. The use of dilute seedings gave a smaller amount of mold in each flask and made the rating more accurate and easier to determine.

Although this method is based upon personal judgment it possesses several distinct advantages because it permits a follow-up check on all cultures throughout the growth period and a good evaluation of different types of mold growth. The first ratings are made when growth first appears and every two or three days thereafter. By this method readings can be made earlier and it is believed that the results are as accurate as they would be if the mass of mold were weighed on a microbalance.

Although readings were made for almost a month, the early readings seemed to be the most important, especially in determining the differences in the culture media. For example, although a crude casein digest with mineral salts and sugar showed, in 15 days, a growth that was comparable with the growth on liquid SABOURAUD'S medium for the same length of time, the growth on the SABOURAUD'S medium was much greater to about the thirteenth day. In fact, the growth of the mold on the casein digest medium did not appear for at least 10 days, while that on the SABOURAUD'S medium showed growth in 7 days. In the various other synthetic mixtures used growth of the mold also appeared later on these media than on SABOURAUD'S medium. This mold was found to produce certain substances which, in other experiments, seem to stimulate its growth. There is the probability that it can also modify amino acids or even synthesize those acids which are necessary for its best nutrition. It has been observed in almost every test that if the growth period is long enough, usually over 20 days, there is a tendency for all cultures to reach about the same level of growth. There is, however, a wide variation in the growth at the time of the first readings.

AMINO ACID REQUIREMENTS

In the culture media commonly used for pathogenic fungi, peptone and carbohydrates have furnished the food material. Such material is, of course, a complex mixture of highly variable composition containing various (and unknown) mineral salts, variable and undetermined assortments of amino acids and other protein cleavage products, and unknown amounts of unrecognized, as well as recognized, vitamin-like constituents. It is clear, however, that the function of such a mixture in the medium, with regard to the furnishing of the proper protein split material, is an important one, especially since it constitutes the only source of nitrogen.

Just as there are certain amino acids which are essential for mammalian growth and maintenance, likewise, from our knowledge of the requirements of certain yeasts and bacteria, there may be certain amino acids essential for the growth of this pathogenic fungus. Some of the lower plants are able to gain their entire source of nitrogen from nitrates, ammonium compounds, and other inorganic sources of nitrogen. The nitrogen requirements of this mold are more complex than the requirements for other lower plants.

It will not grow on a medium containing only inorganic nitrogen, but it will grow in a medium supplied with a suitable organic nitrogen.

In the study of the amino acid requirements the procedure followed was to start with what may be considered the most complex synthetic medium ever used for the study of microorganisms. This medium contained every pure chemical (including the amino acids and nutrilites) which the writers thought might be necessary or of advantage for the growth of the organism. From this medium, which permitted relatively rapid growth, amino acids were eliminated first by groups, and later singly; and the effects of each elimination were recorded.

The medium[1] used, contained in each liter, in addition to the amino acids, the concentrations of which varied somewhat in the different experiments, the following compounds:

Compound	Weight	Compound	Weight
Sucrose	48.00 gm.	$ZnSO_4$	1.0000 mg.
$(NH_4)_2SO_4$	3.00 "	$MnCl_2$	1.0000 "
KH_2PO_4	4.00 "	H_3BO_3	1.0000 "
$CaCl_2 \cdot 2\,H_2O$	0.10 "	$CuSO_4$	0.1000 "
$MgSO_4$	0.25 "	Pantothenic acid	0.0004 "
$FeCl_3$	0.10 mg.	Vitamin B_1	0.0020 "
KI	0.10 "	Inositol	0.0200 "
$ThCl_3$	0.10 "	Crude lactoflavin	0.0100 "

The amino acids used in the medium included glycine, dl-alanine, dl-serine, dl-valine, dl-leucine, dl-isoleucine, l-proline, l-hydroxy proline, l-tryptophane, l-tyrosine, dl-phenylalanine, dl-methionine, l-cystine, l-histidine, l-arginine, l-lysine, l-aspartic acid, l-asparagin, d-glutamic acid. After most of the work was completed the writers obtained from Dr. W. C. Rose a very small quantity of his new beta-hydroxy-alpha-amino butyric acid and were able to determine its place in the nutrition of this mold.

The control of the pH of the medium is important. It was adjusted so that after the addition of the amino acids the pH was 5.5, which is the most favorable value.

The preliminary experimentation involved in eliminating many of the amino acids as effective nutrient factors, was extensive and too complex to be given in detail. The results of a few of the experiments including the amino acids which seemed to be essential to favorable nutrition will be given.

During this detailed study it was found that the amino acids, glycine, cystine, histidine, tryptophane, tyrosine, glutamic acid, methionine, alanine, hydroxy proline and serine, could be omitted individually and collectively

[1] This medium (omitting the last four items) is a modification of a basal yeast medium which has been used extensively in this laboratory. In actual practice this medium was made up double strength so that solutions of amino acids to be tested could be added to it without diluting it beyond the desired point.

without seeming to affect the medium in any way. Omitting isoleucine, valine, or proline individually had practically no effect, but if two or three were omitted simultaneously growth was diminished.

Leucine appeared to be the most important individual amino acid, while beta-hydroxy-alpha-amino butyric acid (ROSE) and aspartic acid seemed next in importance. The effects of arginine, lysine, and phenylalanine were readily detected. Asparagin and aspartic acid seemed to be interchangeable.

The situation is far from simple, however. Concentration of the amino acids may be an important factor. Antagonistic action and lack of balance between different amino acids seem to be evident, and undoubtedly if the cultural conditions (particularly with respect to the amount of seeding used) were different the results would be appreciably modified. It is therefore not possible to differentiate rigorously between those amino acids which are indispensable and those which are non-essential.

Although previous experiments, repeated in various ways in duplicate, showed that leucine, aspartic acid, arginine, lysine, and phenylalanine are among the most essential amino acids, the results given in table I show that a mixture of these five in equal proportions does not constitute a highly favorable mixture, probably owing to a lack of balance. The figures in the table exaggerate the differences, however, because every culture except the blanks showed growth in 10 days. This is possible only with a fairly favorable medium. If any of the media had been extremely unfavorable growth would have been delayed or prevented entirely. The striking effect brought about by the elimination of leucine should be noted. This is in accord with previous experiments.

Tests not directly connected with the study were made to see if the mold synthesizes tryptophane and tyrosine (two amino acids for which distinctive color tests are available) when growing in a medium which is free from these amino acids. The tests showed that, after hydrolysis of the mold, tyrosine (Millon's reaction) and tryptophane (Hopkins-Cole reaction) were both present, thus proving that syntheses had actually taken place.

CARBOHYDRATE NUTRITION

As a rule organisms do not require specific carbohydrates as they do amino acids. In order to determine whether or not this is the case with the organism under investigation the following experiment was performed:

Culture media (containing nutrilites and other compounds) were made up as in the amino acid experiments, except that each 12 ml. of solution contained 50 mg. of an acid digest (pH 5.5) of highly purified casein. To each flask was added 0.5 gm. of one of the sugars indicated in table II.

From the growth on the blank culture, which contained no sugar, it is clear that this mold can grow to maturity without carbohydrate if the proper amino acids, as well as minerals and growth promoting substances, are

TABLE I

EFFECT UPON GROWTH OF MOLD WHEN ONE OR MORE OF FIVE ESSENTIAL AMINO ACIDS ARE OMITTED FROM THE CULTURE MEDIUM

No.	Amino acids in culture	Growth ratings				
		10 days	12 days	16 days	20 days	24 days
1	Leucine, aspartic acid, arginine, lysine, phenylalanine	5	5	6	6	5
2	(5 mg. each*)	5	5	6	6	5
3	Aspartic acid, arginine, lysine, phenylalanine. (Leucine omitted)	1	2	2	1	1
4	(6 mg. each*)	1	2	2	1	1
5	Leucine, arginine, lysine, phenylalanine. (Aspartic acid omitted)	6	6	6	6	7
6	(6 mg. each*)	6	6	6	6	7
7	Leucine, aspartic acid, lysine, phenylalanine. (Arginine omitted)	8	6	7	8	7
8	(6 mg. each*)	8	8	7.5	8	7
9	Leucine, aspartic acid, arginine, phenylalanine. (Lysine omitted)	5	6	7	7	6
10	(6 mg. each*)	5	6	7	6	6
11	Leucine, aspartic acid, arginine, lysine. (Phenylalanine omitted)	9	9	9.5	9.5	9
12	(6 mg. each*)	9	9	9.5	9	9
13	Leucine, lysine, phenylalanine. (Aspartic acid and arginine omitted)	6	6	7	6	6
14	(8 mg. each*)	5.5	6	6.5	6	5.5
15	Leucine, arginine, phenylalanine. (Aspartic acid and lysine omitted)	6	7	7	6	5
16	(8 mg. each*)	6	7	7	6	5
17	Leucine, arginine, lysine. (Aspartic acid and phenylalanine omitted)	3	3	3.5	4	3
18	(8 mg. each*)	3	3	3.5	4	3
19	Leucine, aspartic acid, lysine. (Arginine and phenylalanine omitted)	4	7	8	9	9
20	(8 mg. each*)	4	7	7.5	8.5	9
21	Leucine, aspartic acid, arginine. (Lysine and phenylalanine omitted)	10	10	10	10	10
22	(8 mg. each*)	10	10	10	10	10
23	Leucine, aspartic acid, phenylalanine. (Arginine and lysine omitted)	8	8.5	9.5	10	10
24	(8 mg. each*)	8	9	9.5	10	10
25	Blank (amino acid omitted)	0	0	0	0	0
26	" (" " ")	0	0	0	0	0

* Quantity of each amino acid used in this and the preceding experiment.

present. The comparative growth ratings indicate that this mold can metabolize all sugars tested except lactose. Growth of the mold on the medium containing lactose was about the same as that on the blank, hence it seems safe to infer that this mold does not produce lactase, although it does produce invertase and maltase.

TABLE II

EFFECT UPON MOLD GROWTH OF THE PRESENCE OR ABSENCE OF SUGARS IN THE CULTURE MEDIUM

No.	Sugar in culture	Growth ratings		
		7 days	10 days	15 days
1	Blank (sugar omitted)	5	5	5
2	" (" ")	4.5	5	5
3	d-Glucose	9	9.5	9.5
4	d-Glucose	9	9.5	9.5
5	Sucrose	10	8	7.5
6	Sucrose	9.5	8	7.5
7	d-Fructose	8.5	8.5	8.5
8	d-Fructose	8.5	8.5	8.5
9	d-Mannose	10	10	10
10	d-Mannose	10	10	10
11	Lactose	5	5	5.5
12	Lactose	5	5	5
13	Maltose	9.5	9	9
14	Maltose	9	9	9
15	d-Galactose	9	9.5	9.5
16	d-Galactose	9.5	9.5	9.5

Effect of nutrilites

Experiments using synthetic media showed that a mixture of amino acids, minerals, and a suitable carbohydrate in the culture do not satisfy the requirements of this mold. The nutrilites, which were available and suspected of being effective in stimulating the growth of this organism, were pantothenic acid, a lactoflavin preparation, inositol, and crystalline vitamin B_1.

The method of preparing crude lactoflavin, which was similar to that of KUHN (**1**), follows. A 3500-ml. volume of whey, from which the casein had been removed by the action of rennin, was brought to a pH of 4.7, heated, and the lactalbumin filtered off. The pH was then adjusted to 1 with HCl and the solution stirred with 35 gm. of fullers' earth for 1½ hours. The

fullers' earth was precipitated by centrifuging, stirred with distilled water, and repeatedly centrifuged and washed until the supernatant liquid no longer gave a test for chloride ion. The adsorbed material was eluted by shaking for 1½ hours with a mixture of 157 ml. of water, 39 ml. of pyridine, and 39 ml. of methanol. The resulting elutate was evaporated to dryness on the steam bath, triturated with methanol, and then water was added. This mixture was heated, shaken for an hour on a mechanical shaker, and finally centrifuged. The resulting clear yellow solution was evaporated to dryness and placed in a vacuum desiccator for 1½ hours. The yellow material was next shaken twice with dry ether, and then dried and weighed. The resulting product weighed 0.21 grams.

This weight of material is much greater than that obtained by KUHN, who used a longer process and a similar quantity of whey. The writers have compared the effect of this preparation on *Trichophyton interdigitale* with the effect on it of pure lactoflavin, which was supplied by Dr. RICHARD KUHN of the Kaiser Wilhelm Institut at Heidelberg, Germany. Each had a stimulative effect. This crude lactoflavin, however, also contains a nutrilite which has a very pronounced effect upon certain other pathogenic fungi (**4**).

Table III shows the comparative growths of this mold in the presence of all possible combinations of the four growth stimulants (nutrilites) taken one, two, three, and four at a time. It will be observed that no growth was observed in the blanks, numbers 31 and 32.

The amount of the growth stimulating substance to be used in each culture was decided on the basis of the effects of various concentrations of these substances upon yeasts and molds as observed in this laboratory over a period of years (**8**). In order to simplify the tests, the same amount of a given nutrilite per culture was used throughout all the tests as follows: pantothenic acid, 0.33 micrograms; lactoflavin preparation, 10 micrograms; vitamin B_1, 2 micrograms; and inositol, 20 micrograms.

In addition to the vitamin-like substances indicated in the table, each culture contained the regular amounts of the basal constituents and the following amino acids in the amounts indicated: alanine, valine, glycine, arginine, phenylalanine, and proline, 5 mg. each; asparagin, 15 mg.; leucine, 10 mg.; isoleucine, 2.5 mg.; and lysine, 4 mg.

As stated elsewhere, the first comparative ratings given in the table III are the most significant. When the cultures indicated in this table were allowed to grow for 26 days all (except the blanks which failed to grow) had the same rating. The inference from this is: given one of the four growth promoting substances, this mold is able to synthesize or dispense with the other three nutrilites. There is, of course, the possibility that more than four nutrilites are needed for the best growth. In such a case, it seems that if this mold is given one of the needed nutrilites it has the ability to produce all the others that it needs.

TABLE III

EFFECT UPON GROWTH OF MOLD OF VARIOUS COMBINATIONS OF FOUR NUTRILITES IN THE CULTURE MEDIUM

No.	Pantothenic acid	Crude lactoflavin	Inositol	Vitamin B_1	Growth ratings		
					8 days	13 days	18 days
1	+	+	+	+	9.5	10	10
2	+	+	+	+	10	10	10
3	+	+	+	−	9.5	9.5	9.5
4	+	+	+	−	9.5	9.5	9.5
5	−	+	+	+	9	9	9.5
6	−	+	+	+	9	9	9.5
7	+	+	−	+	9.5	9.5	9.5
8	+	+	−	+	9.5	9.5	9.5
9	+	−	+	+	8	8.5	9
10	+	−	+	+	8	8.5	9
11	+	+	−	−	8.5	9	9.5
12	+	+	−	−	9	9.5	9.5
13	−	+	+	−	2.5	3	9
14	−	+	+	−	5	7	9.5
15	−	−	+	+	8	8.5	9
16	−	−	+	+	8	8	9
17	+	−	−	+	10	10	10
18	+	−	−	+	9	9	9.5
19	+	−	+	−	4	5	8
20	+	−	+	−	5	6	8
21	−	+	+	−	3	5	8
22	−	+	+	−	3	5	8
23	+	−	−	−	3	3.5	5
24	+	−	−	−	3.5	4.5	7
25	−	+	−	−	7.5	9	10
26	−	+	−	−	8.5	9.5	10
27	−	−	+	−	3.5	5	8
28	−	−	+	−	3.5	5	7
29	−	−	−	+	3	4	6
30	−	−	−	+	3	4	6
31	−	−	−	−	0	0	0
32	−	−	−	−	0	0	0

That this mold, like *Aspergillus niger,* produces pantothenic acid is shown by the fact that when it was grown on a pantothenic acid-free medium, it showed a very definite pantothenic acid content, when tested in accordance with the method developed in this laboratory.

Furthermore, there is evidence that during growth this mold produces vitamin B_1 (or something which serves as an equivalent so far as yeast growth is concerned), because the mold grown on a vitamin B_1-free medium contains a factor which supplements pantothenic acid and inositol in stimulating the growth of "old process" yeast (7).

The apparent ability of one of the nutrilites to replace (in a measure at least) the others seems quite inexplicable and out of line with reasonable expectations, because chemically the different substances have practically nothing in common, and in animal nutrition one vitamin is unable to replace another.

It appears from this study that, in the case of both amino acids and nutrilites, the mold (and from the experience of the writers they expect that molds in general resemble each other in this respect) has remarkable latent synthetic capabilities. It is believed that in time, during a long growth period, mechanisms are developed for the production of any one of a number of specific cell constituents which, under other conditions, may be taken from the environment in the form of food, and which are necessary for rapid growth.

Mineral requirements

The writers have attempted to determine which minerals are important for this mold, laying particular stress upon the question of "trace elements." Emphasis must be placed upon the uncertainty which exists with regard to all experiments where minute traces of minerals exert a great effect upon the growth of an organism. The writers can demonstrate that certain ions have an effect upon the mold but they cannot be sure that the effect produced is entirely due to the substance which they intended to add. Even the purest chemicals, which they have used at all times, invariably contain impurities in almost undetectable quantities. These considerations, however, do not prevent the giving of a fairly accurate statement about the mineral requirements of this mold. The effect of impurities has been minimized by using such small amounts of the ions in question that any impurities present have been reduced to an infinitesimal concentration.

The medium, which has been used throughout this study, containing $(NH_4)_2SO_4$, KH_2PO_4, $CaCl_2$, $MgSO_4$, $FeCl_3$, KI, $ThCl_3$, $ZnCl_2$, $MnCl_2$, H_3BO_3 and $CuSO_4$, was taken as the basis of the mineral requirements because it supports an excellent growth of *Trichophyton interdigitale* when supplemented with suitable organic compounds. Owing to the fact that the writers are concerned with growth and not rate of growth, the comparative ratings are not used. In recording growth and no growth, + and − signs respectively are used. The "trace elements" have been divided into two groups: group I containing KI and $FeCl_3$; and group II containing $ThCl_3$, $CuSO_4$, $MnCl_2$, $ZnSO_4$, and H_3BO_3. Table IV shows the effect of omitting the various components of the medium singly and in various combinations.

TABLE IV

EFFECT UPON GROWTH OF MOLD WHEN INORGANIC COMPOUNDS AND MINERALS ARE OMITTED SINGLY OR IN GROUPS

No.	MEDIUM	GROWTH
1	Complete assortment	+
2	KH_2PO_4 omitted	–
3	$MgSO_4$ "	–
4	$CaCl_2$ "	–*
5	Trace elements, group I omitted	–
6	Trace elements, group II "	–
7	KI omitted	+
8	$FeCl_3$ omitted	–
9	$CuSO_4$ "	–
10	$MnCl_2$ "	–
11	$ThCl_3$ "	–
12	H_3BO_3 "	–
13	PO_4— "	–
14	SO_4— "	–
15	Cl- "	+
16	$ThCl_3$ and KI omitted	+
17	$ThCl_3$, KI and H_3BO_3 omitted	+
18	$(NH_4)_2$ SO_4 omitted	–

* In the absence of calcium the mold showed very scant growth in 18 days but it then turned brown and died within two days.

All ions tested except thallium, iodide, chloride, and borate seem to be essential. Excellent growth was obtained in the absence of these ions, but the omission of any other ion resulted in no growth. While phosphate is necessary from a nutritional standpoint, it is also of value as a buffer. Since a base is produced by the growth of the organism, its growth rate is suppressed. The presence of an efficient buffer in the medium enables the growth to continue for a longer period of time than in an unbuffered medium.

Summary

1. The amino acid, carbohydrate, nutrilite, and mineral requirements of *Trichophyton interdigitale* have been studied.

2. This mold cannot grow on a synthetic medium unless it is supplied with certain amino acids. No single amino acid seems to be absolutely indispensable, although leucine seems almost so. Aspartic acid (or asparagin) and alpha-amino-beta-hydroxy-n-butyric acid (ROSE) are very helpful, while proline, valine, lysine, phenylalanine and arginine are less necessary. A varied assortment of amino acids is superior to any group of three or four. However, glycine, cystine, histidine, tryptophane, tyrosine, glutamic acid, methionine, alanine, hydroxy proline and serine seem to have no specific

effect. Tryptophane and tyrosine (and possibly certain other amino acids) are synthesized by the growing mold.

3. *Trichophyton interdigitale* can utilize all of the more common sugars tested except lactose, but mannose gives the best growth.

4. Given an adequate assortment of amino acids, mineral salts, and a suitable carbohydrate the mold studied will not grow unless it is supplied with at least one of the following growth promoting substances: pantothenic acid, inositol, crystalline vitamin B_1, or a lactoflavin preparation. Growth is greatly stimulated by the addition of the four growth promoting substances, although satisfactory growth will result with only one. This mold synthesizes nutrilites as it grows.

5. The inorganic ions which seem to be essential for growth are potassium, ammonium, zinc, magnesium, iron, copper, manganese, calcium, phosphate, and sulphate, while chloride in minute quantities seems to be beneficial. Growth can be maintained over a longer period of time by the use of an abundance of phosphate as a buffer.

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PHOTOPERIODIC RESPONSE OF CERTAIN LONG AND SHORT DAY PLANTS TO FILTERED RADIATION APPLIED AS A SUPPLEMENT TO DAYLIGHT

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(WITH TEN FIGURES)

Introduction

In a previous publication (**4**) it was shown that the red portion of the visible spectrum was especially effective in inducing photoperiodic responses in long day plants when used to prolong short winter days. These responses generally included earlier flowering, increased internodal development, and a greater top-root ratio. The yellow, blue, and green regions of the visible spectrum of wavelengths shorter than 650 mμ were relatively much less effective. It was further indicated that the infra-red was relatively ineffective under the conditions of experimentation. When the white radiation of an incandescent lamp was used to prolong the normal winter daylight period, the responses obtained were like those produced by red radiation (650 to 720 mμ) alone.

Since all of the plants studied in the above mentioned paper were of the long day type, it was considered of interest to determine the spectral region responsible for the vegetative condition induced in typical short day plants as contrasted with the reproductive responses of long day plants, such as occurs when the white radiation of an incandescent lamp is used to prolong the day.

SCHAFFNER (**3**) has shown that corn, which normally produces staminate terminal inflorescence under long summer days, tends to give rise to carpellate flowers in the tassels under the short days of winter, especially when the corn is seeded around the first of November in the vicinity of the latitude of 40° N. Corn might, therefore, be considered a short day plant so far as femaleness in the terminal inflorescence is concerned.

Experimentation

The plant material used in the present investigation included China aster, *Callistephus chinensis* variety Heart of France, sunflower, *Helianthus cucumerifolius* variety Stella, and *Scabiosa atropurpurea* variety Azure Fairy as long day plants; and *Cosmos bipinnatus* variety Double Late Crimson King, *Salvia splendens* variety Drooping Spikes, and *Tithonia speciosa* as typical short day plants. *Zea mays* variety Purdue Bantam was included in the series from the standpoint of sex reversal as effected by wavelength of supplementary radiation.

TABLE I

FLOWERING RESPONSE OF A SERIES OF LONG DAY AND SHORT DAY PLANTS TO VARIOUS WAVE LENGTHS OF SUPPLEMENTAL RADIATION

PLANT MATERIAL	NO. PLANTS	WAVEBAND	TOTAL TIME TO FLOWER	REMARKS
			days	
Callistephus chinensis var. Heart of France, Seeded 8–30, Irradiation begun 10–18	10	white	156	
	10	red	156	
	10	green	156	Plants germinated in 12½-hour day
	10	blue	156	
	10	control	156	
Callistephus chinensis var. Heart of France, Seeded 12–3, Irradiation begun 1–15	30	white	139	
	30	red	139	
	30	green	160	Plants germinated in 9¼-hour day
	30	blue	134	
	30	control	168	
Helianthus cucumerifolius var. Stella, Seeded 12–15, Irradiation begun 1–14	10	white	126	
	10	red	126	
	10	green	153	
	10	blue	128	
	10	control	153	
Scabiosa atropurpurea var. Azure Fairy, Seeded 12–18, Irradiation begun 1–14	10	white	142	
	10	red	142	
	10	green	—*	
	10	blue	—	
	10	control	—	
Salvia splendens var. Drooping Spikes, Cuttings made 8–15, Irradiation begun 10–1	20	white		Plants were clonal cuttings and were in flower when radiation was begun
	20	red		
	20	green		
	20	blue		
	20	control		
Salvia splendens var. Drooping Spikes, Seeded 12–3, Irradiation begun 1–15	30	white	—	
	33	red	—	
	28	green	129	
	31	blue	134	
	22	control	125	
Cosmos bipinnatus var. Late Double Crimson King, Seeded 12–18, Irradiation begun 1–17	5	white	—	
	5	red	—	
	5	green	67	
	5	blue	67	
	5	control	67	
Tithonia speciosa, Seeded 11–17, Irradiation begun 1–15	6	white	—	
	6	red	—	
	6	green	116	
	6	blue	116	
	6	control	116	
Zea mays var. Purdue Bantam, Seeded 12–15, Irradiation begun 1–14	5	white	110	
	5	red	110	
	5	green	110	Carpellate tassel
	5	blue	110	Carpellate tassel
	5	control	110	Carpellate tassel

* All dashes in this column indicate that the plants in the group failed to flower before the close of the experiment on May 20.

The seeds were sown in flats on the dates indicated in table I and the seedlings were transplanted as necessary to 3-inch pots in a rich manure composted loam. They were further transferred to 6-inch pots as the plants became larger and were thus grown to maturity. Two series of aster and salvia plants were grown. The first of each series was started in the fall and the second in the winter. The earlier group of aster seedlings was germinated in a 12½-hour day which has been shown to be of sufficient length to bring aster into flower at the temperatures used (**1**). The later plants were germinated in a 9½-hour day and they remained in the vegetative state in the control plots until the days became sufficiently long to induce reproduction. The earlier group of salvia was originated from a clonal line propagated from cuttings, the first of which were made the previous spring from a single mother plant. At the beginning of the experiment, when the lights were applied on October 18, all the plants were in full flower. The later group of salvia was started from seed and the plants were still vegetative when the lights were applied.

A portion of the plants of the later series of both aster and salvia were harvested for data on dry weight of tops and roots and for leaf area and height at the ages shown in tables II and III. The remaining plants and the other plant material used in the experiment were allowed to come to maturity for flowering data.

TABLE II

RESPONSE OF CHINA ASTER, *Callistephus chinensis* VAR. HEART OF FRANCE, TO VARIOUS WAVE LENGTHS OF SUPPLEMENTARY RADIATION. SEEDED DECEMBER 3, 1934. RADIATION BEGUN JANUARY 15, 1935, MAINTAINING AN 18-HOUR DAY. HARVESTED APRIL 4, 1935. TEMPERATURE 50° TO 55° F.

SUPPLEMENTAL RADITION	NO. PLANTS	DRY WEIGHT			TOP-ROOT RATIO	NO. LEAVES PER PLANT	AV. TOTAL LEAF AREA	AV. HEIGHT	AV. TOTAL TIME TO FLOWER
		TOP	ROOT	TOTAL					
		gm.	*gm.*	*gm.*			*sq. cm.*	*cm.*	*days*
White 1 foot candle	30	3.4	1.4	4.8	2.4	61	323	23.2	139
Red, 650–1400 mμ	30	3.5	1.3	4.8	2.7	62	460	26.6	139
Green, 450–550 mμ	30	2.1	1.2	3.3	1.7	60	288	4.3	160
Blue, 380–510 mμ	30	3.1	1.3	4.4	2.4	63	428	16.7	134
Control short day	30	1.5	1.4	2.9	1.1	63	256	3.2	168

TABLE III

RESPONSE OF *Salvia splendens* VAR. DROOPING SPIKES TO VARIOUS WAVELENGTHS OF SUPPLEMENTARY RADIATION. SEEDED DECEMBER 3, 1934. RADIATION BEGUN JANUARY 15, 1935, MAINTAINING AN 18-HOUR DAY. HARVESTED APRIL 4, 1935. TEMPERATURE 50° TO 55° F.

SUPPLEMENTAL RADITION	No. PLANTS	DRY WEIGHT			TOP-ROOT RATIO	No. LEAVES PER PLANT	AV. TOTAL LEAF AREA	AV. HEIGHT	AV. TOTAL TIME TO FLOWER
		TOP	ROOT	TOTAL					
		gm.	*gm.*	*gm.*			*sq. cm.*	*cm.*	*days*
White 1 foot candle	30	5.0	2.5	7.5	2.0	45	688	40.4	.....
Red, 650–1400 mμ	33	5.0	2.1	7.1	2.4	39	671	44.7	.....
Green, 450–550 mμ	28	5.3	2.3	7.6	2.3	65	662	42.6	129
Blue, 380–510 mμ	31	5.7	2.5	8.2	2.3	53	670	45.6	134
Control, short day	22	5.5	2.7	8.2	2.0	60	527	32.4	125

The same filters for the red, green, and blue plots were used in the present experiments as were described previously (**4**). However, the intensity used was one-fourth as great, due to the fact that in the blue the series was balanced for 500 watts instead of 2000 watts. Figure 1 presents the relative energy distribution curves of the filters as obtained by plotting the product of the transmittancy of the filters and the relative spectral energy distribution of the lamp used as calculated from the data of HOLLADAY (**2**). The filters consisted of dyed gelatin films on glass, the details of preparation of which have not yet been published. The incandescent lamp radiation

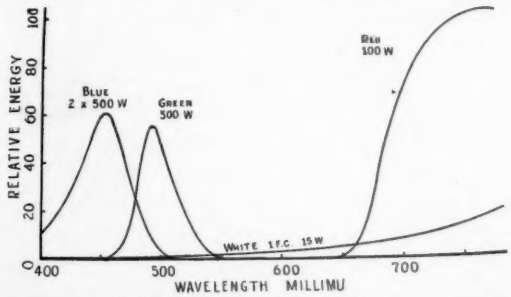

FIG. 1. Relative spectral radiant energy distribution curves for experimental plots as based on calculations of filament temperature of incandescent lamp sources.

was adjusted to an average intensity of one foot candle over the plot, as measured by a Weston illuminometer.

During the night the plots were isolated from each other by black oil cloth and gaberdine curtains in such a manner as not to interfere seriously with the normal circulation of air. The scattered light in the control plots was kept below 0.02 foot candle, as measured by a Macbeth illuminometer.

The artificial radiation was applied from sundown until 6 hours before sunrise, thus maintaining an 18-hour day. The period of lighting was automatically controlled by means of electric time clocks.

The greenhouse temperature was manually controlled at 50° F. night and 55° F. day.

Results

The results secured as to flowering time of the group of plants used are presented in table I. Tables II and III give more detailed data on dry weights, leaf areas, and heights of the long day aster and of the short day salvia plants. Figures 2 to 10 are photographs of the plant material taken at the time of flowering.

FIG. 2. Response of China aster, *Callistephus chinensis* var. Heart of France, when seeded in late summer and germinated under long day conditions of 12½ hours. The natural daylength was supplemented with various wavebands of artificial visible radiation as indicated to maintain an 18-hour day.

The responses of the two sets of aster plants which were varied as to planting time are quite interesting from the point of view of the effect of time of seeding on the subsequent response to daylength as well as to wavelength of supplementary radiation. Those plants which were germinated in the fall in a daylength of 12½ hours and at higher temperatures all flowered at approximately the same time regardless of supplementary radiation. These plants are shown in figure 2 at the time of blooming. Plants which were germinated in winter in days of 9½ hours showed definite reproductive effects owing to the additional radiation of various wavelengths. Those

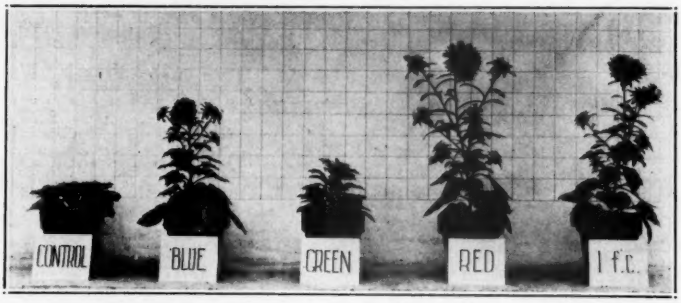

FIG. 3. Response of China aster, *Callistephus chinensis* var. Heart of France, when seeded in early winter and germinated under short day conditions of 9¼ hours. The natural daylength was supplemented with various wavebands of artificial visible radiation as indicated to maintain an 18-hour day.

under the white, red, and blue radiation all flowered at approximately the same time while those under the green and control conditions bloomed 3 and 4 weeks later (fig. 3).

Sunflower and scabiosa both flowered under the full radiation of an incandescent lamp and under red radiation. Sunflower also bloomed under the

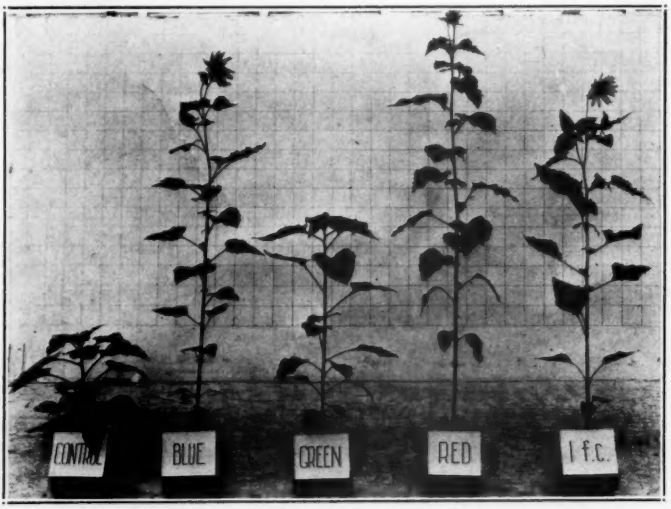

FIG. 4. Sunflower, *Helianthus cucumerifolius* variety Stella, grown under natural short day conditions supplemented by various wavebands of artificial visible radiation to maintain an 18-hour day.

FIG. 5. *Scabiosa atropurpurea* var. Azure Fairy flowered early under days lengthened by red and white radiations.

blue at the same time as under the red, 25 days before those plants under the green and control. Scabiosa had not flowered under the blue, green, or control by the close of the experiment on May 20. Longer internodes occurred under the influence of the red and white radiation than were present in the other groups in all three plants.

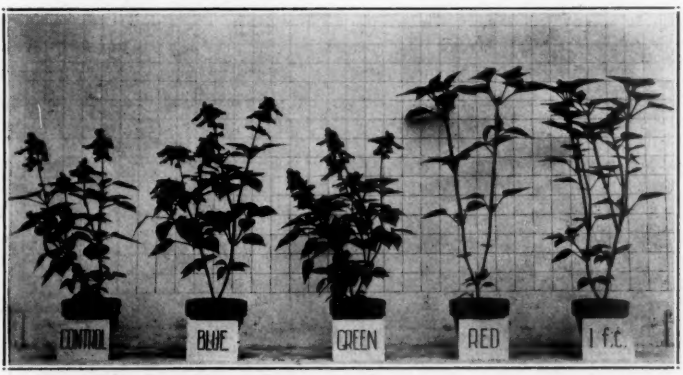

FIG. 6. *Salvia splendens* var. Drooping Spikes grown under short day conditions supplemented by various wavebands of artificial visible radiation as indicated to maintain an 18-hour day. These plants were cuttings representing a single clone. They were all in the reproductive state when lights were applied.

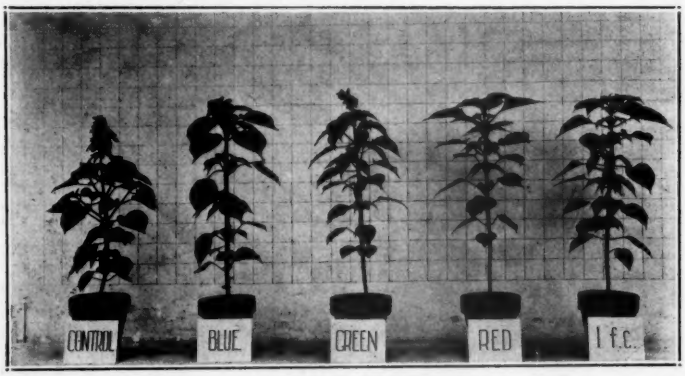

FIG. 7. *Salvia splendens* var. Drooping Spikes treated as indicated in figure 6. Plants from seed sown in early winter. Plants were vegetative when lights were applied.

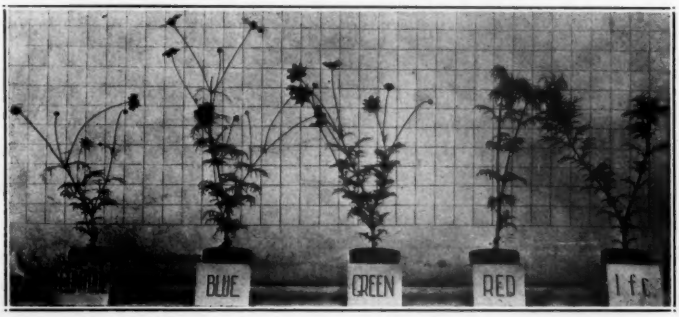

FIG. 8. *Cosmos bipinnatus* var. Late Double Crimson King remained in vegetative condition when the days were lengthened by red and white radiations.

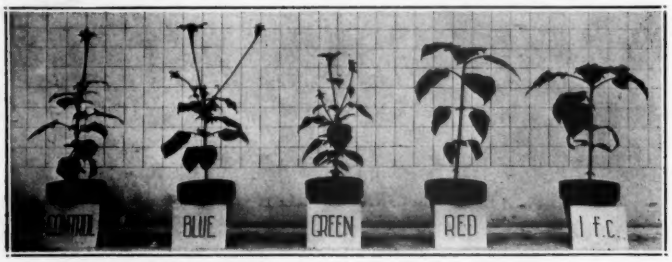

FIG. 9. *Tithonia speciosa* remained in vegetative condition when days were prolonged by red and white radiation.

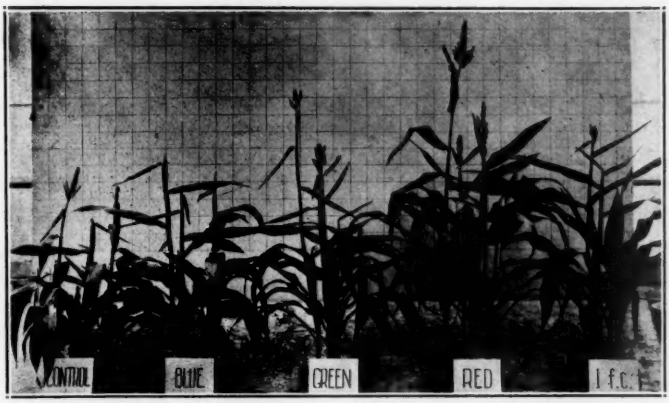

FIG. 10. *Zea mays* var. Purdue Bantam prevented from producing carpellate inflorescences when red and white radiations were used to prolong the days.

The more detailed data presented in table II on aster indicate that the lowest dry weight of tops, the lowest top-root ratio, the smallest leaf area, and the shortest plants occurred under green radiation and in the control, the control value usually being somewhat less than that for the green. The term root is not applied in a strictly anatomical sense but it is used to designate the underground portion of the plant. An increased synthesis of dry matter under red and white light is indicated by these dry weight values. However, most of the products of this increased activity are not translocated to the roots since the plants under all of the experimental conditions had root systems of comparable dry weights. It is interesting to note that the greater leaf surface in the red is associated with increased dry weight. The plants in the red were taller than any of the others. Those in the blue were somewhat shorter than in the red or white and had shorter internodes. No very great differences occurred in the dry weight of tops, total dry weight, or top-root ratios of plants in the white, red, or blue. Little difference was noted in dry weight of roots or number of leaves between any of the aster plants under the various conditions.

None of the short day plants bloomed under the red or white radiation. Salvia, cosmos, and Tithonia all flowered in the green, blue, and control, with no significant difference in time of flowering between any of these three conditions. With the salvia from clonal cuttings which were all in full flower at the time the days were lengthened with the various wavelengths of radiation, those under white and red light discontinued flowering very soon after the application of artificial light while those in the control, green, and blue remained in the reproductive state. With those seeded in winter, and

apparently vegetative when the days were lengthened as designated, only those in the green, blue, and control bloomed.

With salvia, the lowest dry weight of tops and total dry weight occurred in the white and red. The blue and control conditions gave the greatest top and total dry weight. Green radiation produced plants with intermediate values for these weights. The control plants had the smallest total leaf area and were the shortest. No large differences could be noted in height or leaf areas between plants from the other experimental conditions. The weight values are indicative of a decreased synthesis of dry matter due to days prolonged with red and white light. These are directly contrasted with the results of the long day aster.

In the case of corn, red radiation was capable of preventing the production of carpellate inflorescences in the tassel, which have been shown by SCHAFFNER to appear in short days and as occurred in the present investigation in the control group of plants. The addition of blue or green radiation had no effect in preventing the appearance of such inflorescences.

The responses obtained in the present investigation indicate that the red region of the visible spectrum, of wavelengths longer than 650 mμ, is capable of inducing a reproductive condition in long day plants and of inducing a vegetative condition in short day plants when it is used to lengthen normally short winter days. The same region is also capable of producing longer internodes in the vegetative portions of both long and short day plants. With short day species such as cosmos and Tithonia, the over-all height of the plants in flower under blue and green radiation may appear greater than that of the non-flowering plants in the red due to the lengths of the flowering stems.

Green radiation had little effect with the species used other than to increase the size of the plant somewhat. This is true for both long and short day plants.

Blue radiation does not appear to produce the definite results occurring with the red. The long day aster and sunflower bloomed under the blue about as readily as under the red, although the plants under the blue were neither so tall nor, in the case of aster, did they synthesize so much dry matter. With scabiosa, no flowering occurred although the plants were larger than in the green or control. On the other hand, the short day plants behaved very much as they did under the short days of the control.

Although both aster and sunflower are long day plants, the minimum daylength required to induce the reproductive state is relatively short. The aster plants seeded late under days prolonged by blue, red, and white light bloomed just 20 to 30 days sooner than those plants under the short day. This indicates that the natural daylength was probably suitable for blooming at the time those plants under the red flowered, but that the control

plants had not matured quite so rapidly. Indications are that the natural daylength was nearing a critical one for both sunflower and aster.

Aster is a plant which approaches the indifferent type in response. It has been shown (**1**) that the Heart of France, a variety of China aster is capable of flowering at any season of the year when grown at relatively high temperatures of 65° to 70° F. It has been shown further (**5**) that at has been shown (**1**) that the Heart of France, a variety of China aster, is flower in days shorter than 11 to 12 hours, irradiation periods lengthening the days to 21 hours for only 40 days during the seedling stage were capable of inducing the reproductive condition. Low intensities (**5**) of 0.1 foot candle applied under similar conditions are also capable of inducing reproduction. Thus it appears that aster was always very close to the threshhold of the vegetative condition in the control plots and only a small stimulus was required to induce reproduction. Although less detailed work has been done with the sunflower, the present results indicate that it falls into the same class.

The minimum daylength necessary for flowering in scabiosa is much longer than for aster and sunflower as indicated in the present work. Further, scabiosa has been found by the authors, in unpublished work, to require much higher intensities of supplementary light for blooming than aster and sunflower, at the temperatures and daylengths used. Thus, under the conditions of the experiment, the natural daylength at no time approached a critical one for scabiosa. It should be kept in mind that no attempt was made to regulate the natural daylength during the course of the experiment and that this decreased or increased according to the season.

For the short day plants, no critical natural daylengths apparently occurred during the duration of the investigation.

From the energy distribution curves shown in figure 1, it can be seen that the blue effect on aster and sunflower must have been due to energy of wavelengths shorter than 450 mμ since the green radiation had slight effect and the filter used in the green transmitted energy up to this point.

It might be inferred that red radiation longer than 650 mμ is that portion of the visible spectrum most effective in producing photoperiodic effects, both in inducing flowering responses in long day plants and in inducing a vegetative condition in short day plants. There seems to be a maximum peak of response in the neighborhood of 650 to 700 mμ (**4**) and possibly a second lesser peak at wavelengths shorter than 450 mμ.

In addition to such contrasting reproductive effects with long and short day plants, red radiation of low intensities causes an increased synthesis of dry matter in long day plants and a decreased synthesis in short day plants when it is used to produce relatively long days during the winter months.

Summary

An investigation is reported concerning the effects of three bands of radiation used to prolong normal winter days to 18 hours on the photoperiodic responses of three long day plants, China aster, *Callistephus chinensis* variety Heart of France, sunflower, *Helianthus cucumerifolius* variety Stella, and *Scabiosa atropurpurea* variety Azure Fairy; and of three short day plants, *Salvia splendens* variety Drooping Spikes, *Cosmos bipinnatus* variety Late Double Crimson King, and *Tithonia speciosa*. The wavebands include the red from 650 to 1400 mμ, the green from 455 to 550 mμ, and the blue from 380 to 510 mμ. The effect of these radiations on the sex reversal of *Zea mays* variety Purdue Bantam is given. The results obtained with the full radiation of an incandescent lamp totaling 1 foot candle of energy with the seven species listed above are included. The plants were grown under greenhouse conditions and received the full winter daylight of the duration and intensity naturally available at that season of the year. The results obtained and the conclusions drawn are as follows:

1. China aster, sunflower, and Scabiosa, long day plants, bloomed early under days prolonged with red and white radiation. Aster, seeded in winter, produced greater dry weights, both of tops and total dry weight, under the red, indicating a greater synthesis of dry matter. That this added material was not translocated is shown by a comparison of the dry weights of the roots of the plants under all of the experimental conditions. Blue radiation induced earlier flowering in aster and sunflower but not in scabiosa. Aster and sunflower failed to flower as quickly under green radiation and control conditions as under the red. Scabiosa had not bloomed under the blue, green, or control conditions by late spring.

2. Salvia, cosmos, and Tithonia remained in the vegetative condition when the days were lengthened with red and white radiation. They flowered in short days and days lengthened with blue and green light. Red radiation caused a decrease in the dry matter synthesized.

3. Aster seeded in the fall under long day conditions of 12½ hours failed to make any increased reproductive response when the days were lengthened as designated, the plants in all the plots blooming at approximately the same time. When germinated in a shorter day of 9½ hours, the response is as indicated above.

4. Salvia from clonal cuttings, with all plants in flower when placed under days lengthened by wavebands indicated, reverted to the vegetative state under red and white radiation. They remained in the flowering condition under the other treatments.

5. Corn, which produces carpellate inflorescences in the tassel under short days, was prevented from producing such inflorescences by the red and white radiation used to prolong the day but not by the blue or green.

6. Conclusions are drawn that, under the conditions of the experiment, red radiation is most effective in producing the photoperiodic response both in long and short day plants; that some especially sensitive plants such as aster respond to the blue radiation; and that green radiation has little effect when used to prolong the day.

The authors wish to express their appreciation to Professor LAURENZ GREENE who offered valuable criticism during the course of the work, and to ALICE P. WITHROW who assisted in the planning of the experiment and preparation of the manuscript.

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CHANGE IN ACTIVITY OF ENZYMES, SOLUBLE CARBOHYDRATES, AND INTENSITY OF RESPIRATION OF RICE SEEDS GERMINATING UNDER WATER

P. S. Erygin

(WITH THREE FIGURES)

Introduction

In the world market upland rice (grain) occupies a very modest place, since its production is comparatively limited, and harvests are uncertain since they depend upon precipitation. For this reason upland rice cannot compete with lowland rice. Upland rice is used exclusively in those regions possessing insufficient water for irrigation purposes. Camus (**2**) identifies four types of rice. Of these, the type requiring least water is "Caingin" while that having the highest water requirement is "typical lowland." In spite of this classification, the two types do not possess definite characteristic qualities, so that certain "upland" varieties of rice grow very poorly on soil containing only ordinary amounts of water but grow very vigorously under irrigated conditions.

There is little literature relating to upland rice, especially in relation to its physiological character. Among the latter is to be found the work of the following investigators: Inouye (**4**), Gile and Carrero (**3**), Jiro Onodera (**5**), Villegas (**9**) and Nagaoka (**7**). The conclusions of these investigators indicate the existence of well defined differences between upland and lowland rice.

The present work presupposes a biochemical and physiological study of the first stages of development of the rice plant, namely, swelling and germination of seeds. The writer will here deal with the metabolism of soluble carbohydrates, changes of dry matter, activity of enzymes, and respiration. In these experiments seeds of the lowland variety Oobe and upland variety Bely were used. The seeds of these varieties were germinated under two sets of conditions: on filter paper, and under water. The stages of germination were: (1) air-dry seeds; (2) swollen seeds; (3) sprouting seeds; (4) seeds with stems 2 cm. in length; (5) seeds with stems 3 cm. in length; (6) seeds with stems 10 cm. in length.

Observations

Dry matter and hygroscopicity

Rice seeds lose a greater part of their weight when germinated in water than when germinated on filter paper. Undoubtedly this is due to the fact that the easily soluble substances are diffused into the surrounding water

medium (fig. 1). The change in quantity of dry matter in germinating seeds is closely related to the change in its hygroscopicity. Minimal hygro-

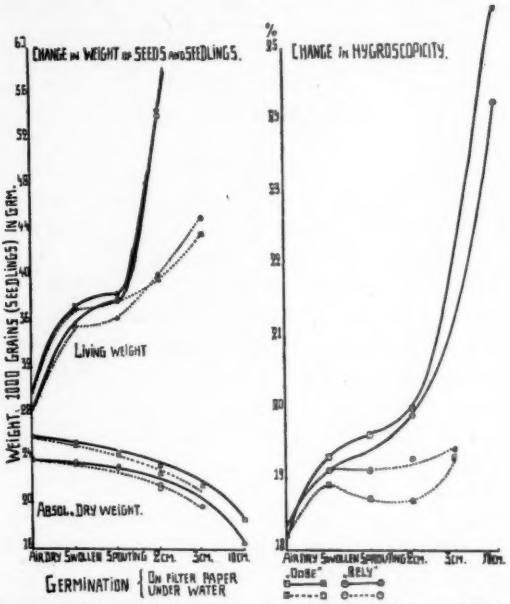

FIG. 1. Changes in the weight and hygroscopicity of Oobe and Bely rice seeds and seedlings, when germinated on filter paper, and under water.

scopicity is observed in air-dry seeds while maximum values are observed in seedlings 10 cm. in length.

Germination is accompanied by the formation of substances which possess to a marked degree the property of swelling. Quantitatively and qualitatively the composition of the colloidal plasm changes with growth; simultaneously the relationship between these colloids and water is changed.

SOLUBLE CARBOHYDRATES

By means of hydrolysis the starch stored in the rice seed is changed first into maltose and then into glucose. The formation of maltose is generally regarded as a necessary intermediate step in this process. At the present time, however, it has been established that α-glucoside bonds do not exist in the starch molecules. In these experiments the writer could not discover a relationship between the accumulation of maltose and the hydrolysis of starch at germination. Possibly the starch is changed directly into glucose without the formation of maltose. Literature on this subject mentions the

TABLE I

SOLUBLE CARBOHYDRATES DURING GERMINATION OF RICE SEEDS

(MG GLUCOSE IN 100 GRAINS)

CONDITION OF MATERIAL	GERMINATION ON FILTER PAPER				GERMINATION UNDER WATER			
	MONO-SACCHA-RIDES	SACCHA-ROSE	MALTOSE	TOTAL SOLUBLE CARBOHY-DRATES	MONO-SACCHA-RIDES	SACCHA-ROSE	MALTOSE	TOTAL SOLUBLE CARBOHY-DRATES
Variety Oobe								
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Air-dry	8.33	94.00	0	102.33	8.33	94.00	0	102.33
Swollen	1.76	3.18	0	10.94	2.28	5.81	0	8.09
Sprouting	66.00	6.00	0	72.00	48.33	27.77	33.33	109.4
Stems 2 cm. in length	.....	.....	.....	.....	9.11	6.00	3.35	18.4
Stems 3 cm.	.....	.....	.....	.....	180.88	3.33	0	184.2
Stems 10 cm.	328.57	95.71	0	424.28	.....	.....	.....	.....
Variety Bely								
Air-dry	7.00	35.62	0	42.62	7.00	35.62	0	42.62
Swollen	1.33	3.51	0.07	4.91	1.65	2.88	5.38	9.91
Sprouting	35.55	44.44	0	79.99	47.21	28.33	0	75.56
Stems 2 cm. in length	.....	.....	.....	.....	10.00	1.68	16.00	27.68
Stems 3 cm.	.....	.....	.....	.....	35.33	15.58	0	50.91
Stems 10 cm.	112.5	14.37	0	126.87	.....	.....	.....	.....

possibility that hydrolysis of starch may proceed so rapidly that maltose does not accumulate in significant quantities. The writer believes that such a condition exists when the maltase enzyme shows greater activity than amylase. On the other hand it is necessary to take into consideration the possibility of the breaking down of starch directly into glucose.

Rice seeds germinated on filter paper (aerated) showed little or no formation of maltose. Likewise maltose is absent in upland rice seeds. With germination under water, however, a more or less significant quantity of maltose is formed, up to the development of a stem 2 cm. in length. Before and after these periods the presence of maltose is not noted (table I).

In view of the fact that the activity of the maltase enzyme is comparatively weak and shows no change (table II), the dynamics of maltose formation may be explained not as a result of the breaking down of starch but rather as a synthetic process. This synthesis may be explained by the fact that the activity of saccharase is greatly weakened in the rice seeds germinated in water. Therefore this enzyme is unable to change monosaccharides into saccharose and as a result maltase is able to act on these sugars. In this case the maltose is a synthetic product; that is to say, starch breaks down without the formation of maltose.

In dry (ungerminated) rice seeds the soluble carbohydrates exist almost exclusively as saccharose. Particular attention should be given to the fact that monosaccharides and saccharose are to be found in dry seeds. The variety Oobe contained 102.3 mg. of soluble carbohydrates while Bely contained only 42.6 mg. in a like quantity of seed (100 grains). The marked decrease in soluble carbohydrates during the swelling of the seeds (at germination) indicates the important rôle these sugars play in the life activities of the seed (when it germinates) in water. Undoubtedly the presence of insufficient oxygen supply, where soluble carbohydrates exist in any quantity, facilitates the development of the seed sprouts. From this point of view rice seeds having a store of large molecule carbohydrates possess less ability to germinate in water.

Enzymes

In view of the fact that enzymes are the most sensitive products of cell activity that may be used as indicators of the intensity of the biochemical processes of the living organ, the writer studied the activity of enzymes in the period of germination in water.

Our general knowledge of the dynamics of enzymes in seeds is based on the systematic investigations conducted by Oparin (**8**). His work related to the activity of oxidation and hydrolytic enzymes in dry and germinating seeds (**1, 8**).

In rice seeds and seedlings the following enzymes were found: amylase, maltase, saccharase, and catalase.

TABLE II

CHANGE IN ACTIVITY OF ENZYMES

(GLUCOSE PER 100 GRAINS PER 20 HOURS)

ENZYME	VARIETY	CONDITIONS OF GERMINATION	SEEDS			SEEDLINGS		
			AIR-DRY	SWOLLEN	SPROUTING	2 CM.	3 CM.	10 CM.
			mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Amylase	Oobe	On filter paper	6.69	6.51	30.55	93.83	.....	167.57
		Under water	6.69	4.76	30.40	76.82	134.01	.....
	Bely	On filter paper	33.80	36.27	98.42	95.71	.....	148.08
		Under water	33.80	30.01	69.70	92.89	144.48	.....
Maltase	Oobe	On filter paper	4.67	2.68	6.44	9.59	.....	12.62
		Under water	4.67	2.56	10.05	8.88	6.72	.....
	Bely	On filter paper	1.02	5.24	9.56	11.28	.....	14.32
		Under water	1.02	1.02	8.68	10.67	8.00	.....
Saccharase	Oobe	On filter paper	7.93	12.43	12.31	47.05	.....	102.01
		Under water	7.93	8.85	11.74	13.54	20.25	.....
	Bely	On filter paper	8.57	11.28	12.47	48.13	.....	63.68
		Under water	8.57	8.66	9.98	18.03	17.83	.....
Catalase (cm.³ oxygen per 100 grains per 5 minutes	Oobe	On filter paper	7.8	19.3	49.9	227.7	.....	664.6
		Under water	7.8	11.3	27.3	49.5	59.3	.....
	Bely	On filter paper	8.4	8.9	44.2	154.9	.....	380.7
		Under water	8.4	7.8	19.2	55.3	72.6	.....

The change in activity of amylase is shown in table II and figure 2. The curve of this graph verifies results of former investigations that lack of oxygen limits the increase of amylase. Activity of this enzyme in submerged germinating seeds is less than that in unsubmerged seeds as long as the leaves do not emerge from under water. Under submerged conditions the activity of amylase decreases to the same degree in both lowland and upland varieties.

The activity of maltase in seeds germinating on filter paper and in water varies little. In the rice seed maltase is very weak and during germination it increases but slightly. In this regard it should be noted that the quantity of maltase present in rice seeds is significantly less than that of amylase.

Air-dry seeds of both varieties contained large quantities of saccharase. This apparently is connected with the presence of cane sugar. During the swelling of seeds as well as during the succeeding stages of germination, enzyme activity increases with unequal rapidity. Seeds germinating in water contain less saccharase, apparently because of a lack of oxygen for its formation. Insufficient aeration and accompanying conditions first of

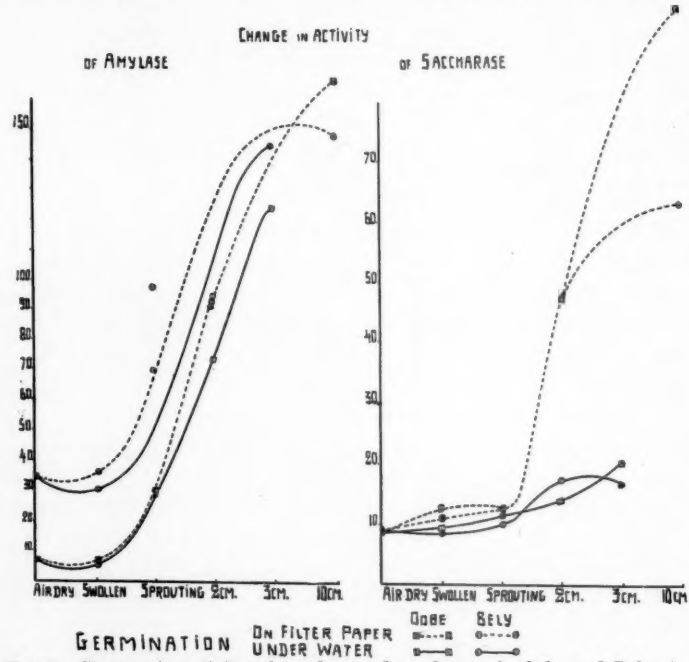

Fig. 2. Changes in activity of amylase and saccharase in Oobe and Bely rice seeds and seedlings germinated on filter paper, and under water.

all check the formation of new or fresh saccharase. Figure 2 shows the repressive effect of submergence on saccharase in germinating rice seeds.

Air-dry seeds contained insignificant quantities of catalase, which is generally regarded as a sign of full maturity. Figure 3 illustrates the dependence of catalase on the oxygen supply. Germination in water is accompanied by a marked repression of catalase in both varieties. Reduction of activity begins in both varieties at germination. During the period of formation of seed leaves, the activity of catalase in seedlings grown in water is less than one-half that of seedlings grown on filter paper. When leaves 2 cm. in length appear, the difference in activity reaches 460 per cent. in the case of Oobe and approximately 300 per cent. in the case of Bely. From

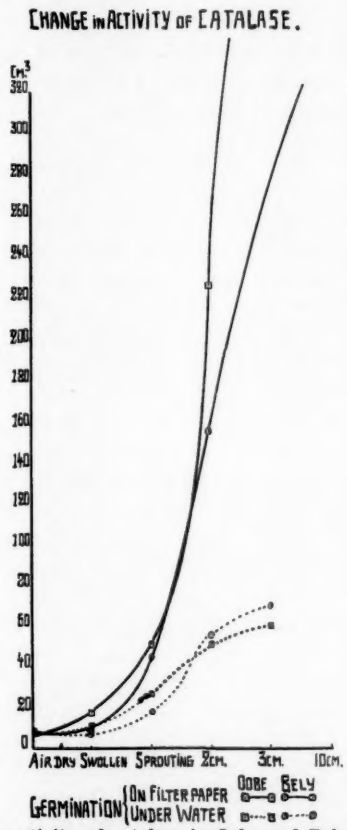

FIG. 3. Changes in activity of catalase in Oobe and Bely rice seeds and seedlings when germinated on filter paper, and under water.

this it will be seen that the intensity of respiration of these plants likewise decreases. Such a parallelism between the respiration process and activity of catalase indicates the close relationship of this enzyme to the oxidation process. Relationship between quantity of catalase and intensity of respiration has been established by many investigators (**6**). On this basis catalase in rice seeds may be regarded as a true indicator of the intensity of the reactions accompanying growth. Data concerning the activity of enzymes show the absence of significant differences in the activity of the enzyme complexes of lowland and upland varieties. (From the view-point of the writer, however, this does not negate the possibility of the existence of lowland and upland varieties of rice with respective specific properties.)

Respiration

Under normal aeration, seeds and seedlings of both varieties respired almost equally. For example, seedlings with leaves 2 cm. in length of Oobe absorbed[1] in the course of 24 hours 25 cm.^{3} and Bely 28 cm.^{3} of oxygen (table III).

In the absence of aeration, intensity of respiration is reduced to a significant degree. Plants of such growth but growing in water absorb in the case of Oobe 4.6 cm.^{3} and Bely 2.7 cm.^{3} of oxygen. This difference in oxygen absorption is observed in seedlings during the entire period of their growth in water. Just as soon as the first leaf appears above water, immediately stronger absorption of oxygen is noted. Differences in this regard are to be noted between various varieties. The lowland variety Oobe increases its oxygen absorption 10 per cent. in comparison with the previous under-water stage, while Bely shows an increase of 97 per cent. It is assumed that under water the upland seedlings are unable to absorb sufficient oxygen for their life processes and as a result large quantities of unoxidized products such as ethyl alcohol and various aldehydes may be found. These products, during oxidation (respiration), require a larger quantity of oxygen than the volume of CO_2 given off during this process. The presence of these large quantities of unoxidized products in submerged upland rice seedlings accompanies the marked depression of their respiration process. The lowland variety does not show this depression.

Judging from the quantity of CO_2 produced, respiration decreases when shoot development begins, that is, during the later stages of germination. This is indicated also by the improved absorption of oxygen (table III).

It should be noted that of the two indicators of respiration the quantity of liberated CO_2 shows a greater respiration intensity than the oxygen absorption during the entire period of submergence of the plant.

In view of the fact that respiration measurements were made of both O_2

[1] Measurement of respiration was conducted with a gasometer type of respirator.

TABLE III

INTENSITY OF RESPIRATION DURING GERMINATION OF RICE SEEDS

(GAS PER GM. DRY MATTER PER HOUR)

CONDITION OF MATERIAL	ABSORBED O_2				PRODUCED CO_2			
	OOBE		BELY		OOBE		BELY	
	ON FILTER PAPER	UNDER WATER	ON FILTER PAPER	UNDER WATER	ON FILTER PAPER	UNDER WATER	ON FILTER PAPER	UNDER WATER
	*cm.*³	*cm.*³	*cm.*³	*cm.*³	*cm.*³	*cm.*³	*cm.*³	*cm.*³
Air-dry	0.00033	0.00033	0.00041	0.00041	0.00038	0.00038	0.00048	0.00048
Swollen	0.028	0.010	0.037	0.014	0.055	0.045	0.064	0.054
Sprouting	0.049	0.029	0.044	0.021	0.097	0.087	0.080	0.061
Stems 2cm. in length	1.060	0.195	1.192	0.115	1.060	0.208	1.192	0.133
Stems 3cm. " "	.....	0.214	.....	0.227	.....	0.198	.....	0.113
Stems 10cm. " "	.....	0.861	.....	0.691	.....	0.728	.....	0.449

and CO_2 simultaneously, we are able to prove that the respiratory quotient very seldom is equal to unity in spite of the fact that large quantities of carbohydrates were present in the plants. Generally the respiratory quotient was greater than unity. Seeds germinated in water show higher values than those germinated on filter paper. Both the value and the degree of change of the respiratory quotient indicate differences between lowland and upland varieties of rice (table IV).

TABLE IV

CHANGE OF RESPIRATORY QUOTIENT OF SEEDS AND SEEDLINGS

PLANTS	CONDITIONS OF GERMINATION	SEEDS			SEEDLINGS		
		AIR-DRY	SWOLLEN	SPROUTING	2 CM.	3 CM.	10 CM.
Barley	On filter paper	1.00	1.00	0.87	0.96	.....	.....
	Under water	1.03	1.00	1.40	1.08	.....	.....
Rice, upland var. Bely	On filter paper	1.17	1.74	1.81	1.00	.....	.....
	Under water	1.17	3.86	2.91	1.15	0.50	0.67
Rice, lowland var. Oobe	On filter paper	1.15	1.96	1.98	1.00	.....	.....
	Under water	1.15	4.50	3.00	1.06	0.92	0.84
Rice millet	On filter paper	1.00	1.75	1.99	0.88	.....	0.85
	Under water	1.00	5.91	13.49?	1.07	.....	.....

During germination in water upland rice increases its respiratory quotient less than the lowland variety. The writer assumes that upland rice lacks the ability to draw on other sources of energy (anaerobic respiration) as a supplement to normal respiration. This may be considered as generally true for all starchy seeds growing under ordinary conditions of soil moisture.

To verify this supposition, the writer determined the respiratory quotient of barley and rice millet. The former may be considered as accustomed to ordinary moisture conditions while the latter is able to grow in water with as much facility as rice. The experimental conditions were the same as those for rice. When germinated in water, barley showed a respiratory quotient equal to unity. On the third day, an increase of the quotient to 1.40 was noted, after which death resulted. The seeds of rice millet germinated and had a respiratory quotient always greater than unity.

The maximum values of the respiratory quotient which were observed in seeds germinating in water were as follows: rice millet + 5.91, lowland rice + 4.50, upland rice + 3.86, and barley + 1.40. It is possible to use the respiratory quotient values as a basis for the identification of lowland and upland varieties of rice.

Summary

1. Germination of rice seeds under water is accompanied by greater loss of dry matter and change in properties of colloidal substances than germination on filter paper. In addition, the volume of the seedling itself increases more rapidly on the filter paper than under water.

2. Soluble carbohydrates are found in greater quantities in the dry seeds of lowland varieties than in those of upland varieties. The main forms of soluble carbohydrates in the rice seeds are saccharose and monosaccharides. Maltose is found during certain stages of germination under water.

3. In general, enzyme action is suppressed in seeds germinating under water especially in the case of saccharase and catalase. This begins from the time of swelling of the seed and continues through the germinating period.

4. Respiration intensity of seeds of Oobe and Bely varieties, germinated on filter paper, is practically equal. When germinated under water, seeds of both varieties respired considerably less intensely. The lowland variety Oobe absorbs oxygen more freely under water than the upland variety Bely.

5. The respiratory quotient for both lowland and upland varieties when germinated on filter paper is approximately the same, but differs markedly in the case of seeds germinated under water. In the latter case the respiratory quotient is always greater than unity.

RICE EXPERIMENT STATION
KRASNODAR, U. S. S. R.

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EFFECTS OF CARBON ARC LIGHT ON THE CHEMICAL COMPOSITION AND VEGETATIVE PROPAGATION OF TOMATO PLANTS GROWN WITH A LIMITED SUPPLY OF NITROGEN[1]

JOHN W. MITCHELL
(WITH THREE FIGURES)

When succulent plants growing under favorable conditions of light, temperature, and moisture, are deprived of nitrogenous fertilizer their chemical composition and growth habits generally change. These responses usually include an increase in the carbohydrate content, particularly in the case of the polysaccharides, followed by a decrease in moisture content and percentage of total nitrogen, which is especially noticeable in the soluble nitrogenous compounds (**5, 8, 9**). Vegetative growth is retarded, particularly in the above-ground portion, the new leaves produced are small and light green in color, and fruiting is often checked when the plants become stiff and woody.

In propagating many varieties of plants vegetatively, cuttings often produce roots more quickly and grow more vigorously when taken from the "harder" portion of a plant or when made from a plant that exhibits the above characteristics. Typical symptoms of nitrogen deficiency and carbohydrate accumulation are not easily obtained in tomato plants by limiting the external nitrogen supply when they are grown during the dark cloudy periods of winter or early spring. This is due mainly to the small amount of carbohydrate synthesized under these conditions. Various changes in the growth and chemical composition of tomato plants occur when they are transferred from natural illumination of low intensity during the winter to artificial illumination from a carbon arc lamp of somewhat greater intensity and grown with a nitrogen-free nutrient. Root production by stem cuttings from tomato plants under such conditions are discussed.

I. Chemical composition of plants grown in arc light

METHODS

Cuttings made from tomato plants (*Lycopersicum esculentum*) in the fall were planted in quartz sand contained in 6-inch clay pots and supplied with nutrient solution containing nitrate nitrogen as described by NIGHTINGALE (**7**). Following this application of nutrient, the sand was kept moist with

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tap water and an occasional application of nitrogen-free nutrient solution. The light intensity in the greenhouse was relatively low during most of the time, as cloudy weather prevailed for approximately 60 per cent. of the daylight hours. After the plants had grown several inches in height, the nitrogen was washed from the sand, nitrogen-free nutrient added, and the plants moved into a room where their only source of light was 12 hours' daily illumination from a carbon arc lamp which has been described (**6**). The intensity of visible light supplied by the arc varied between 900 and 1000 foot candles at the surface of the leaves. Measurements were made of the total radiant energy in light from the carbon arc by means of a thermopile recording potentiometer system. The total radiant energy in natural light in the greenhouse was also measured by the same means for one week in December prior to transferring the plants to artificially controlled conditions. Results based on the area of curves drawn by the recording potentiometer show that the average total energy recorded per hour of natural light during the week was only 11.5 per cent. of the energy recorded per hour in arc light. The natural daily period of illumination recorded was about 8 hours while the daily period of illumination under artificially controlled conditions was 12 hours. The total radiant energy to which the plants were exposed each day under artificially controlled conditions was about 13 times more than they had received each day during the previous week in the greenhouse. The quality of light produced by the carbon arc closely approximated the quality of natural light in which plants were grown (**3, 4**). A temperature of 68 to 69° F. was maintained during periods of illumination and 71 to 72° F. during darkness. The relative humidity of air in the room varied between 50 and 75 per cent.

Composite samples consisting of stems, petioles, and leaves, and containing 150 to 200 gm. of tissue were collected for chemical analyses at the same hour on the first, fifth, and tenth day during the interval in which the plants were exposed to arc light. The fresh and dry weight of each sample was determined, rapid drying being accomplished by means of a well aerated oven at 75° C. The dry material was pulverised and stored in air-tight containers for analysis. The methods used for the determination of reducing sugars, starch and dextrin, nitrate, and total nitrogen have been described by NIGHTINGALE (**8**). The usual microchemical tests for starch and nitrates were made on fresh tissue at frequent intervals throughout the experiment.

EXPERIMENTAL RESULTS AND DISCUSSION

At the time the plants were placed in artificial light there were 6 to 8 leaves on each stem. These leaves were large, and dark green in color. The stems were green, slender, and not stiff to the touch. Analyses of the plants

showed the water content of the stems and leaves to be relatively high (table I). The amount of carbohydrate present, including reducing sugars,

TABLE I

ANALYSES OF THE STEMS AND A COMPOSITE SAMPLE CONTAINING THE LEAVES AND PETIOLES OF TOMATO PLANTS GROWN IN ARTIFICIAL LIGHT WITH NITROGEN FREE NUTRIENT SOLUTION. RESULTS ARE EXPRESSED ON DRY WEIGHT BASIS, EXCEPT MOISTURE AND DRY MATTER

MATERIAL	SAMPLE	INITIAL	5 DAYS	10 DAYS
		%	%	%
Stems	Moisture	90.2	89.7	89.4
	Dry matter	9.8	10.3	10.6
	Nitrate nitrogen	0.58	none	none
	Total nitrogen	1.22	0.91	0.87
	Reducing sugars	5.13	5.20	6.01
	Sucrose	trace	7.13	9.00
	Starch and dextrin	4.12	18.90	21.17
	Total carbohydrates	9.25	31.23	36.18
Leaf blades and petioles	Moisture	89.7	89.6	88.2
	Dry matter	10.3	10.4	11.8
	Nitrate nitrogen	trace	none	none
	Total nitrogen	2.95	2.71	2.68
	Reducing sugars	3.78	6.16	5.19
	Sucrose	none	3.17	4.21
	Starch and dextrin	2.07	16.12	18.91
	Total carbohydrates	5.85	25.45	28.30

sucrose, starch, and dextrin was small, as the plants had grown for several weeks in December in a period of low light intensity caused by dark cloudy days. The percentage of total nitrogen present was moderately high, as the plants had been supplied with a limited amount of nitrate nitrogen. Microchemical tests showed the presence of a small amount of starch in the lower fifth of the stems. Nitrate nitrogen was found in all parts of the plants, although the amount present in the leaves and petioles was not sufficient to be detected by the methods used in macro-analyses. The area of xylem, as shown by transverse mid-stem sections, was small in comparison with the area of the pith region, which is a condition characteristic of succulent tomato plants (5).

When these plants were given a minus nitrogen nutrient solution and exposed to arc light 12 hours daily for a period of 10 days, there was a marked change during the first 5 days in both their chemical composition and outward appearance. Microchemical tests, made three days after placing the plants under arc light, showed nitrate nitrogen to be lacking in the upper

half of the stems. Starch had accumulated in the endodermis and in the pith cells adjacent to the protoxylem in the central portion of the stems. The stems were noticeably stiffer than on the first day.

At the end of 5 days of illumination with arc light, two or three lower leaves on each plant had turned yellow, the remaining leaves had turned to a light green color, and the sides of the stems nearest to the light source were bluish purple. There was a definite increase in the amount of lignification in the stems which, in part at least, accounted for the stiffness of the stems.

Table I shows that the percentage of dry weight of the stems and leaves increased slightly during the first 5 days the plants were exposed to arc light. There was a noticeable increase in the percentage of carbohydrates in the aerial portion of the plants which was very evident in the amounts of non-reducing sugar and polysaccharides present. The percentage of total nitrogen in the stems and leaves decreased, and on the fifth day no nitrate nitrogen was detected by analyses of the stems, leaves, or petioles.

After the plants had been given 12-hour exposures to arc light for 10 days, the stems of the plants were stiff, and bluish purple in color on the side nearest the light source. Few new leaves had been produced, and the plants grew very little in length during the experiment. Three or four lower leaves on each plant had turned yellow, while the remaining leaves were grey green with the exception of the very young leaves at the tip of the stem which showed no appreciable change in color. There was little change in the quantity of roots during the time the plants were exposed to artificial light.

Analyses showed that the carbohydrate content of the stems and leaves increased between the fifth and the tenth day of the experiment. Transverse sections taken from the middle of several stems showed a marked thickening of the walls of lignified xylem cells, and a greater portion of the total area of the section was occupied by lignified xylem tissues (fig. 1). The aboveground portion of the plants contained a lower concentration of total nitrogen than on the fifth day of exposure to artificial light. Nitrate nitrogen was lacking, or present in such small amounts that it could not be detected by the analytical methods used (table I). Microchemical tests also showed that nitrate nitrogen was not present in any part of the plants in quantities which could be detected. Starch had accumulated in appreciable quantities in the pith, cortex, and medullary rays of the lower half of the stem and was easily detected in sections taken up to within three inches of the stem tip (fig. 1).

In summarizing these results it is apparent that the total carbohydrate content of the stems increased most rapidly during the first half of the ten-day period, during which the plants were given minus nitrogen nutrient and

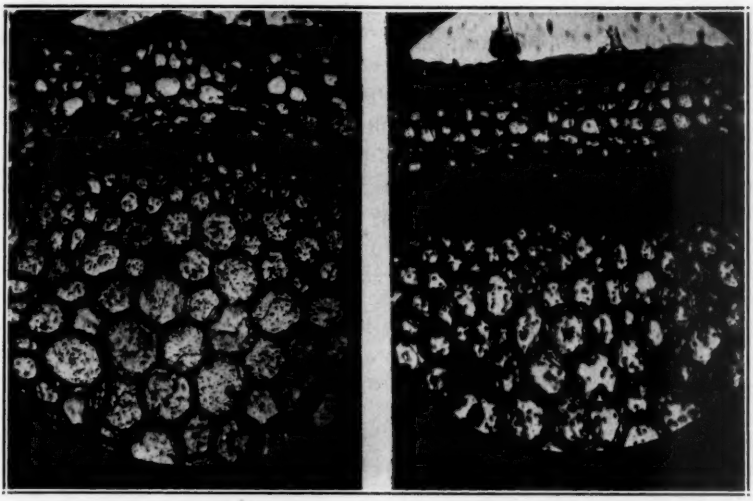

FIG. 1. Photomicrographs showing relative amounts of starch and lignified xylem tissues in transverse mid-stem sections from plants before (on left) and after irradiation for 10 days with arc light.

grown in arc light (fig. 2). The greatest increase in the total carbohydrate content was also noted in the leaves and petioles during the first 5 days of exposure to artificial light. Secondary thickening of the xylem, which was most apparent during the first half of the 10-day period, was associated with the rapid accumulation of carbohydrates in the plants. The storage of carbohydrates continued from the fifth to the tenth day but the rate of accumulation was less rapid. The abscission of older leaves, which accompanied the decrease in the rate of carbohydrate accumulation in the plants during the period of nitrogen starvation, resulted in a decrease of 40 to 60 per cent. in the number of expanded leaves per plant. This reduction in the amount of photosynthetic tissue was closely associated with, and may have in part accounted for, the decrease in the rate of carbohydrate accumulation during the period in which the plants were grown in artificial light.

The concentration of reducing sugars remained nearly constant in the stems of the plants subjected to arc light and a limited nitrogen supply, while there was a marked increase in the amount of sucrose, dextrin, starch, and other polysaccharides, associated with cell thickenings. The concentration of reducing sugars in the leaves and petioles increased from about 2 to 6 per cent. of the dry weight of these parts in the first 5 days and remained at approximately this concentration during the remainder of the experiment. On the other hand, sucrose, which was present in very small

quantities when the total carbohydrate concentration of the plants was low, showed a continuous increase during the experiment similar to that of the polysaccharides.

The percentage of total carbohydrate in the above-ground portion of tomato plants grown in arc light for 10 days with a limited nitrogen supply was approximately the same as that of plants grown in natural light during the spring, summer, and fall with a similar nutrient supply (**1, 5**). It should be recognized, however, that the time required for the accumulation of appreciable amounts of carbohydrates in tomato plants is by no means constant. It depends upon several factors including the quality and intensity of light (**1, 2**), day length, external nitrogen supply, and the amount of inorganic nitrogen in the plant that is available for new protein synthesis at the beginning of the period during which all external sources of nitrogen are withheld.

Root production by cuttings from irradiated plants

Method and results

In view of the fact that relatively high carbohydrate or "mature" plants are often desirable for the purpose of vegetative propagation, (**2, 10, 11, 12**), experiments were carried out to determine whether a short exposure to light from a carbon arc lamp would affect the rooting of cuttings made from the stems of tomato plants following a period of irradiation. Succulent plants grown during the winter in well fertilized soil were washed free of soil, transplanted to quartz sand, and supplied with nitrogen-free nutrient. A number of these plants were left in the greenhouse at a temperature of 56 to 70° F. under natural light from an overcast sky. The maximum daily intensity varied between 500 and 1100 foot candles but much lower intensities prevailed during a large part of each day. The remainder of the plants were given continuous exposure to light from a carbon arc lamp and grown at the same air temperature. The intensity of the artificial light was approximately 1200 foot candles measured by means of a Weston light meter. A comparison has already been made between the quality of the arc light and the quality of natural light that prevailed during these experiments. At the end of 40 and 80 hours, stems cut at the ground level from the irradiated plants and also from those exposed to natural light, were supported on glass rods over wet cellucotton in moist chambers. Microchemical tests showed practically no starch in the plants grown under natural light after 40 or 80 hours, while those in artificial light had accumulated starch in the pith cells although there was an abundance of nitrogen in all parts of the plants. Half of the stem cuttings taken from plants grown under natural light produced roots, and at the end of a week or ten days all of the stems from plants grown under these conditions, including those that rooted, had decayed

and were discarded. Root growth on stems taken from the plants which had been illuminated 40 hours continuously with artificial light was not appreciably different from that of the controls, and the tissue decayed in approximately the same length of time. The stems from plants which were exposed continuously for 80 hours to artificial light all produced roots, and after 10 days only one stem had decayed at the apical end.

In a repetition of these experiments, some plants were grown in the greenhouse under natural light of low intensity, while others were exposed for 80 hours to artificial light at intervals of about 12 hours daily. Less than half of the stems of control plants grown in the greenhouse produced roots, and all but one of these cuttings turned dark and decayed within ten days (fig. 3). On the other hand, all of the cuttings made from the stems of

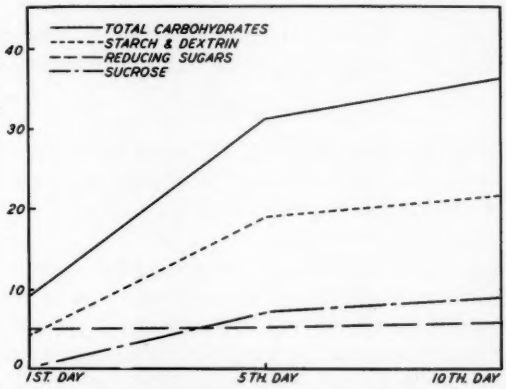

FIG. 2. Total carbohydrate, starch and dextrin, reducing sugars, and sucrose expressed as percentage of dry matter in the stems of tomato plants which were given daily exposures to arc light and nitrogen free nutrient for a period of ten days.

irradiated plants produced within 48 hours roots which grew vigorously during the following two weeks in a moist chamber kept in the laboratory. Microchemical tests showed that starch accumulated in the plants given artificial light during the period of irradiation. A decrease in the amount of starch in the cuttings accompanied the growth of roots, as no starch could be detected in them at the end of two weeks. The cuttings from irradiated plants were discarded after 15 days at which time there was no evidence of decay, some of the roots had reached a length of 3 or 4 inches, and several cuttings had produced new stem growth at the nodes. It is evident from these experiments that root growth by cuttings made from the stems of tomato plants low in carbohydrates was favored by exposing the plants to

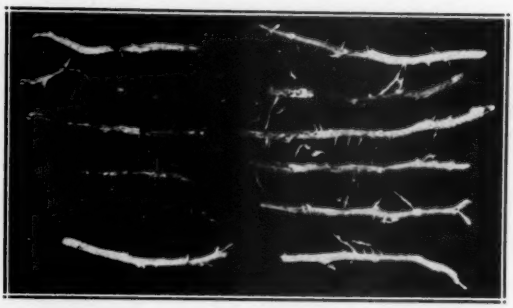

FIG. 3. Right row, roots developed by tomato cuttings from plants grown in natural light of low intensity; left row, from plants exposed for 80 hours to arc light at 12-hour daily intervals prior to making the cuttings.

artificial light of the kind used in these experiments prior to making the cuttings, and that the irradiation made the cuttings less susceptible to decay.

Summary

1. The total carbohydrate content of the above-ground portion of tomato plants increased approximately 400 per cent. during a 10-day period in which the plants were grown with a nitrogen-free nutrient and exposed 12 hours daily to light from a carbon arc lamp.

2. In plants given a limited supply of nitrogen and illuminated with artificial light daily, sucrose, starch and dextrin, and polysaccharides concerned in the thickening of cell walls, accumulated rapidly at first, then more slowly after several days of illumination, while the percentage of reducing sugars changed very little during the experiment. This decrease in the rate at which carbohydrates accumulated in the plants was accompanied by yellowing and abscission of the older leaves thus decreasing the total leaf area per plant. This reduction in photosynthetic tissues may have been in part responsible for the decreased rate of carbohydrate accumulation during the latter part of the period in which the plants were grown in artificial light.

3. There was an increase in the production and growth of roots by cuttings made from tomato plants grown in natural light of low intensity after the plants were exposed to arc light prior to making the cuttings. Cuttings made from such irradiated plants were less subject to decay than those taken from plants grown in natural daylight of low intensity during winter.

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RESPONSE OF QUACK GRASS TO DEFOLIATION AND FERTILIZATION[1]

S. T. Dexter

Introduction

Studies of the response of many species of forage plants to various cutting and fertilization treatments have brought out certain general principles. In general, short and frequently repeated defoliation has been found to result in decreased root reserves and decreased yields of foliage. Heavy fertilization with nitrogen usually stimulates top growth, and is associated with decreased root growth. Frequent cutting in immature stages has been found to result in slower recovery after cutting. Rather extensive reviews of the literature pertaining to root reserves have been made by Thomas (**8**), Graber (**1**), Harrison (**2**), and others.

Quack grass, (*Agropyron repens*) is generally considered a noxious weed (**3**), particularly notable and objectionable because of its extreme vegetative persistence and its pronounced tendency to spread by means of rhizomes.

The most superficial consideration of the attributes and the reputation of this species makes it evident that it is not easy to exhaust its reserves of organic food, nor to produce in it a condition in which it is extremely susceptible to injury by defoliation. The present paper describes experiments designed to study the response of this species to various degrees of defoliation and nitrogenous fertilization. The behavior induced in the above-ground parts of the plant as well as the condition of the rhizomes were studied.

Experimentation

Greenhouse experiments

On February 20, 1935, sods were obtained in the field and weighed samples of rhizomes were planted in sand in the greenhouse. Certain of the cultures were supplied with a nutrient solution high in mineral nitrogen (plus nitrogen cultures), while others were supplied with a solution that contained very small amounts of nitrogen (minus nitrogen cultures). Three series of experiments were carried out. The cultures in series I were completely defoliated whenever growth reached a height of 1 inch. Those in series II were cut after about 4 weeks of growth, when the leaves were 5 or 6 inches long. Those in series III were permitted to grow for 6 weeks without defoliation. After 6 weeks in the greenhouse, all cultures were overturned

[1] Contribution from the Section of Farm Crops, Michigan Agricultural Experiment Station, East Lansing, Michigan. Journal article no. 258 (new series).

in such a way that the plants were covered by about 6 inches of sand, in order to simulate plowing.

In series I plants (frequently defoliated) supplied with plus nitrogen nutrient made more foliar growth than plants supplied with minus nitrogen nutrient for a period of about 3 weeks, after which time the minus nitrogen plants were more vigorous. At the time of the simulated plowing, growth had practically ceased, no new rhizomes had formed, the original rhizomes were flabby and brown and they were dead at the ends. Identical total numbers of culms developed and were harvested from the two sets of cultures. The total dry weight of the plants harvested from the plus nitrogen cultures was about 8 per cent. more than that from the cultures low in nitrogen. Under these conditions, which permitted very limited photosynthesis, the dry weight of the harvested foliage was about 35 per cent. of the dry weight of the rhizomes originally planted. After the cultures were overturned to simulate plowing, no new shoots appeared above the soil surface.

In series II, no new rhizomes had formed, either at the time of cutting after 4 weeks of growth or at the time of simulated plowing, in either plus or minus nitrogen cultures. In an interval of two weeks after simulated plowing, many new shoots were formed in all pots of this series, but none penetrated to the surface of the soil. No difference could be observed between plus and minus nitrogen cultures in this regard.

In series III (not defoliated for 6 weeks), more foliage was produced by the plants of the plus nitrogen than by those of the minus nitrogen cultures, whereas the latter produced more roots and more new rhizomes than the former. It may be well to emphasize that the cultures that had abundant nitrogen produced new rhizomes freely, although they did not occur in as great numbers nor did they have as great weight as those with a limited nitrogen supply. The total dry weight and nitrogen content of the plants in the plus nitrogen cultures were decidedly greater than those of the minus nitrogen cultures.

After simulated plowing, many new shoots reached the surface in all pots, neither plus nitrogen nor minus nitrogen cultures showing superiority. The formation of new rhizomes and the vigorous recovery from simulated plowing would suggest that the old rhizomes of plants grown in both plus and minus nitrogen cultures were high in stored carbohydrates. Foliage and new rhizomes produced in plants of plus nitrogen cultures were in all cases higher in percentage of nitrogen and lower in percentage of dry matter than corresponding material from minus nitrogen cultures.

On February 20, other samples of rhizomes were placed between blotters in a seed germinator at 25° C., in the dark. New growth was cut from these at weekly intervals for a period of about 8 weeks. At first, considerable apical dominance was in evidence, but within 2 weeks, sprouts appeared at

all nodes. At the end of 4 weeks, none of the rhizomes were dead, but some were making very slow growth. Some sheets were watered with tap water, and others with nutrient solution containing mineral nitrogen, but there was no observable difference in growth between them. At the end of 8 weeks, 35 per cent. were entirely dead. An additional 40 per cent. of the rhizomes were described as "not growing," while 25 per cent. appeared able to grow slightly. At this time, rhizomes from the germinator were placed in sand at a depth of about 1 inch. From 20 rhizomes, 5 of which had apparently living buds at the nodes, only 2 leaves finally appeared above-ground, and these were about the width of a coarse bristle. At the time that many of the rhizomes failed to make further growth on the germinator pads, they were, as a rule, bright in color, firm and waxy, and, in general, otherwise indistinguishable from other rhizomes that continued to grow. No conclusions could be reached as to why certain rhizomes in apparently healthy condition should fail to sprout.

Field experiments

Experiments similar to those conducted in the greenhouse were carried out in the field. Plats were laid out in two locations. Those at East Lansing were on a fertile black loam; those at Lake City, about 150 miles north of East Lansing, were on a sandy loam. Ammonium sulphate was added to the plats at East Lansing at the rates of 0, 400, and 800 pounds per acre. At Lake City, ammonium sulphate was applied at the rates of 0, 200, 400, and 600 pounds per acre, and some of the plats received phosphate and potash in addition. The fertilizers were applied early in the spring.

In all cases, ammonium sulphate induced foliar growth greater than that on the unfertilized plats. The growth on sod, receiving 600 or 800 pounds per acre, was extremely heavy, and in all cases the foliage of plants receiving nitrogen was higher in percentage of nitrogen than that of the unfertilized checks.

At intervals throughout the spring, samples of sod were removed from each of the various plats for examination. The percentage of dry matter in the rhizomes was determined. Samples of rhizomes were placed in the germinator to sprout. The sprouts were collected, counted, weighed, measured, and dried. Collections were taken weekly until sprouting virtually stopped. The amount of nitrogen in both sprouts and residual rhizome material was determined in some cases. The date and amount of formation of new rhizomes was determined in the various plats. Part of the data from the plats at East Lansing is shown in table I. All figures in the tables are reduced to the basis of a 10-gm. sample of fresh rhizomes.

Table II shows data obtained sprouting rhizomes which were dug from

TABLE I

DATA ON SPROUTING QUACK GRASS RHIZOMES GROWN IN BLACK LOAM AND FERTILIZED WITH AMMONIUM SULPHATE AT THE RATES OF 0, 400 AND 800 POUNDS PER ACRE, RESPECTIVELY

DATE PUT IN GERMINATOR	PERCENTAGE DRY MATTER IN FRESH RHIZOMES			DRY WEIGHT OF RHIZOMES AFTER SPROUTING			TOTAL DRY WEIGHT OF NEW GROWTH			AVERAGE
	AMMONIUM SULPHATE			AMMONIUM SULPHATE			AMMONIUM SULPHATE			
	0	400	800	0	400	800	0	400	800	
	%	%	%	gm.	gm.	gm.	gm.	gm.	gm.	gm.
April 9	42.4	42.4	42.4	3.36	3.36	3.36	0.243	0.243	0.243	0.243
" 23	38.66	36.49	31.46	3.43	3.21	3.34	0.195	0.25	0.180	0.208
" 30	37.64	34.53	36.14	3.39	3.48	3.09	0.168	0.128	0.183	0.160
May 14	35.99	33.26	31.68	3.48	3.60	3.26	0.064	0.076	0.167	0.102
" 21	.....	.....	.....	3.85	3.63	3.54	0.112	0.105	0.127	0.115
" 28	.....	.....	.....	3.88	3.53	3.56	0.097	0.130	0.064	0.097
Average	37.43	34.76	33.16	3.61	3.49	3.36	0.127	0.137	0.164	.....

sandy soil at Lake City. Each figure is the average, reduced to a 10-gm. sample basis, of four 40-gm. samples of fresh rhizomes.

TABLE II

DATA ON SPROUTING QUACK GRASS RHIZOMES GROWN IN SANDY LOAM AND FERTILIZED WITH AMMONIUM SULPHATE AT THE RATES OF 0, 200, 400, AND 600 POUNDS PER ACRE, RESPECTIVELY. GERMINATED AT 25° C. IN DARK

DATE PUT IN GERMINATOR	NUMBER OF NEW SHOOTS IN FIVE WEEKS				DRY WEIGHT OF NEW SHOOTS				DRY MATTER LEFT IN RHIZOMES AFTER SPROUTING			
	AMMONIUM SULPHATE				AMMONIUM SULPHATE				AMMONIUM SULPHATE			
	0	200	400	600	0	200	400	600	0	200	400	600
					gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
May 27	18	.....	38	.....	0.09	.....	0.25	.....	.....	.....	.....	.....
June 6	4.9	23.5	33.5	40.2	0.021	0.129	0.163	0.200	4.38	4.15	3.76	3.69

The data in table II show the same trends as those in table I. The rhizomes that were dug from heavily fertilized soil made vigorous growth in the germinator. The contrast between fertilized and unfertilized plats is less distinct on the rich soil at East Lansing than it is on the sandy soil at Lake City. In each case, the foliar growth in the field was greater and the content of dry matter in the rhizomes was less in the plants grown in the plus nitrogen nutrients than those grown in the minus nitrogen nutrient.

In spite of this apparent drain upon the storage in the rhizomes, the rhizomes of plants dug from fertilized soil have a much greater ability to sprout in the germinator. As in the experiments conducted in the winter, many rhizomes that appeared to be in perfectly healthy condition failed to sprout. This was particularly notable in the case of plants grown in the minus nitrogen nutrient. Table II shows that, of the samples collected on June 6, those plants that received 600 pounds of ammonium sulphate per acre produced approximately 10 times the number and 10 times the dry weight of new shoots that the rhizomes produced which had been dug from unfertilized soil. So far as their appearance was concerned, there appeared to be no reason why the rhizomes grown in the minus nitrogen nutrient should not sprout as well as the rhizomes grown in the plus nitrogen nutrient. The analyses for dry matter content show that the rhizomes grown in the minus nitrogen nutrient were higher in dry matter than those grown in the plus nitrogen nutrient.

The rhizomes which provided the data for table III were dug at Lake City on June 6. The amount of nitrogen in the original rhizomes, the amount harvested in new shoots, and the amount remaining in the rhizomes after sprouting was virtually complete, are shown in the table. The percentage of nitrogen in the leaves harvested for hay is included to show how the composition of the entire plant was affected. Data are included from plats receiving complete fertilizer (400 pounds per acre of 0–14–6 in addition to the ammonium sulphate) as well as from plats receiving ammonium sulphate only.

Table III shows that a large amount of nitrogen was stored in the rhizomes dug from soil that was high in nitrogen and that, at the same time, foliage that was high in nitrogen was produced on these plants. The plus nitrogen rhizomes placed in the germinator produced sprouts containing from 10 to 20 times the quantity of total nitrogen found in the sprouts from rhizomes dug from unfertilized soil. Although the rhizomes dug from fertilized soil, both before and after being placed in the germinator, were lower in dry matter than those from unfertilized soil, they sprouted much more freely than the minus nitrogen rhizomes. This superior vigor of sprouting continued for 5 weeks, since the production of sprouts continued proportionately greater during the last weeks of the test. It would appear in this case that the ability to sprout was limited more by nitrogen supply than by other factors, and that even the plus nitrogen plants were high in stored carbohydrates. That is, quack grass stored excess nitrogen, as shown by the analysis of the rhizomes, but also developed a superior ability to sprout. From examination of the analyses of the leaves cut for hay, it would appear that still heavier fertilization with nitrogen would probably have given foliage with an even higher nitrogen content. It is of

TABLE III

NITROGEN* IN FRESH RHIZOMES, SHOOTS, RHIZOMES AFTER SPROUTING, AND IN HAY. PLANTS FERTILIZED WITH AMMONIUM SULPHATE AT RATES OF 0, 200, 400, AND 600 LB. PER ACRE, RESPECTIVELY. ALL FIGURES REDUCED TO THE BASIS OF 10 GM. OF FRESH RHIZOMES

FERTILIZER TREATMENT	NITROGEN IN ORIGINAL RHIZOMES				NITROGEN HARVESTED IN NEW SHOOTS				NITROGEN LEFT IN RHIZOMES AFTER SPROUTING				PERCENTAGE PROTEIN (N × 6.25) IN DRY LEAVES HARVESTED AS HAY			
	AMMONIUM SULPHATE				AMMONIUM SULPHATE				AMMONIUM SULPHATE				AMMONIUM SULPHATE			
	0	200	400	600	0	200	400	600	0	200	400	600	0	200	400	600
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	%	%	%	%
N (only)	15.46	18.60	30.16	43.76	0.37	2.53	4.25	6.48	14.86	17.58	23.34	34.90	13.92	16.00	18.22	22.03
P, K, and N	13.68	18.60	17.90	23.30	0.23	2.43	1.96	3.66	13.88	16.10	17.58	21.82	14.75	18.45	19.07	22.5

* Analyses for nitrogen were made by the section of Agricultural Chemistry, Michigan Agricultural Experiment Station.

some interest to observe that the rhizomes of the grass receiving a complete fertilizer stored less nitrogen than those of the grass fertilized with nitrogen alone. This may be due to the fact that the grass grown on the soil receiving a complete fertilizer produced a greater weight of foliage which was, in addition, somewhat higher in nitrogen than foliage from plants grown on soil receiving only ammonium sulphate. It was observed that rhizomes from soil receiving a complete fertilizer tended to rot more quickly in the germinator than those from soil receiving only nitrogen fertilizer.

Rhizomes dug and placed in the germinator on May 27, were in better condition on August 6, so far as ability to sprout and freedom from decay were concerned, than similar rhizomes dug and placed in the germinator June 6. Table I indicates an explanation for this condition. Rhizomes dug early in the spring were found to be far more able to sprout and less subject to decay than those dug at later dates.

New rhizomes, cut from old rhizomes, to about the end of May, made no further growth and rotted when placed in the germinator. Those cut after this date made considerable growth in the germinator. The formation of new rhizomes seemed about equally abundant on heavily fertilized and on unfertilized plats. It was noted, however, that rhizome formation was very scanty in dense sods and abundant in thin sods, regardless of fertilization.

Eradication

Plats of grass at East Lansing, some unfertilized and some receiving 800 pounds of ammonium sulphate per acre, were cut at various dates throughout the spring. Shortly after cutting, the sod was spaded to a depth of about 8 inches, in such a way that the sod was overturned rather than standing on edge. New growth, therefore, had to penetrate approximately 8 inches of soil. When new leaves extended about 4 inches above the surface of the soil, they were cut off with a hoe.

The difficulty of eradication was very great in plants cut and spaded May 13. Grass that was cut on May 13 and again on May 27, and then spaded, was much easier to eradicate by hoeing than that cut only on May 13; but was not observably different from that cut only on May 27. Grass cut on June 11, just before heading, was easier to eradicate by spading and hoeing than any other. Grass cut on June 29 was next easiest, whereas that cut on July 13, when the seed was almost ripe, was very difficult to eradicate. This change in difficulty of eradication corresponds with other records (**3**). Table I shows that the old rhizomes were relatively unable to sprout on June 11. At that time the new rhizomes were not sufficiently mature for vigorous vegetative extension.

The fertilized grass made the most rapid initial growth, after spading, on

all plats, regardless of the date of cutting. After repeated defoliations, however, the fertilized grass appeared to become weakened to such an extent that the unfertilized grass made somewhat more top growth. This corresponds with observations in the greenhouse, but seems somewhat in contradiction to the results obtained from rhizomes grown on the germinator pads. The plus nitrogen rhizomes grown on the germinator pads were always superior to the minus nitrogen rhizomes in ability to sprout, even after weeks of continued removal of new sprouts. Possibly these unfertilized plants were able to obtain nitrogen from the soil for later growth, by which time the high nitrogen plants may have exhausted their carbohydrate supply by vigorous initial growth. The differences between rhizomes grown in fertilized and unfertilized soils obtained from East Lansing were never particularly great, however, even on the germinator pads.

Summary

1. Frequently repeated complete defoliation of quack grass prevented rhizome formation and killed the plants, regardless of fertilization treatment. Under these conditions of defoliation, plus nitrogen plants tended to produce more growth in the first stages and less in the later stages of exhaustion than minus nitrogen plants.

2. Rhizomes taken from plants supplied with plus nitrogen nutrient and with minus nitrogen nutrient at various times in the spring and early summer, and then placed in a seed germinator, progressively decreased in ability to sprout as compared with samples collected in the very early spring. Plus nitrogen rhizomes sprouted more freely than did minus nitrogen rhizomes. These shoots were longer, heavier, and contained a higher percentage of nitrogen than shoots from minus nitrogen rhizomes, but were lower in the percentage of dry matter.

3. Plus nitrogen rhizomes, remaining after sprouting had ceased, were higher in nitrogen and lower in dry matter than those from minus nitrogen plants. Plants receiving nitrogen only stored more nitrogen in the rhizomes than those receiving phosphorus and potassium in addition to nitrogen.

4. When quack grass sod, fertilized heavily with ammonium sulphate, was cut and spaded at various times in the spring and early summer, renewed growth was always more rapid and more vigorous than on adjacent unfertilized plats. After such growth was hoed off several times, the grass in the unfertilized plats seemed somewhat more persistent than that on the highly fertilized, but not markedly so.

5. New rhizomes formed in approximately equal abundance in fertilized and unfertilized sods, but new rhizome formation was very scanty in dense sods as compared with thin sods.

6. Quack grass responds to nitrogenous fertilization by producing

greater top growth which is higher in nitrogen than grass from unfertilized plats.

7. Under the conditions of heavy fertilization with nitrogen in field plats and in the greenhouse cultures, no plants were produced that could be considered low in carbohydrates. Storage, as evinced by formation of new rhizomes and by ability of the old rhizomes to sprout vigorously, occurred in even the most highly fertilized series.

Further experiments are in progress relating to the adaptability of fertilization and cutting practices to the control and eradication of this plant, and to its use in the production of forage.

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NITRITE AND FORMALDEHYDE FORMATION IN CERTAIN ALGAE

ANNA L. SOMMER

(WITH FOUR FIGURES)

I. Photochemical reduction of nitrate to nitrite within the algal cell

For a number of years there has been disagreement on the question as to whether or not light plays an important part in the reduction of nitrate to nitrite by the green plant. Some investigators, including NIGHTINGALE (**12, 13, 14**), THOMAS (**16**), ECKERSON (**7, 8, 9, 10**), MUENSCHER (**11**), and ANDERSON (**4**), have presented evidence that some reduction may take place without light. In much of the work the presence of nitrate-reducing microorganisms was not ruled out. Moreover, in some of the experiments the reducing action was studied by testing for nitrite formed by adding nitrate to the expressed juice of plants. This procedure brings about contact between nitrates and other compounds that might not occur in the intact plant. A method which will detect the presence of, and an increase in, nitrite in a short period of time and without destroying the plant would give more conclusive evidence. It is believed that such a method was used in the work presented in this paper.

MATERIALS AND METHODS

The ALLISON (**1, 2, 3**) magneto-opic apparatus was employed in these experiments, since it was shown by the writer and co-workers (**15**) that by this means formaldehyde could be detected and the amount estimated within small and sufficiently transparent algal cells. Because the method is relatively new and not in general use, a brief explanation of it will be given.

The ALLISON magneto-optic apparatus detects liquids or substances in solution without change in these substances. It is only necessary that sufficient light be transmitted through the tube containing the liquid or solution to make the necessary observations. BISHOP, DOLLINS, and OTTO (**6**) have shown that when a calibrated circle is attached to the analyzing nicol quantitative determinations may be made. The circle readings vary directly with the concentration.

Preliminary experiments showed that the algal suspensions must be dilute or only a few readings could be made before the concentration of the nitrite formed was greater than could be represented by angle readings. It was also found necessary to use light of very low intensity, usually about 0.1 international foot candles. When conditions for nitrite formation by the algae were poor and the light intensity high, higher readings were sometimes obtained for solutions from which the algae had been filtered than for solu-

tions containing algae.[1] Readings were never as high for the filtered as for the unfiltered solutions when these conditions were good, but the differences were always smaller at higher than at low light intensities. The procedure finally adopted for these experiments was as follows: A dilute algal suspension (mixed culture in a solution containing nitrate) was placed in the observing tube and the highest angle at which one of the minima of magnesium nitrite[2] (11.03) could be seen was determined to within 0.5°. A similar reading was obtained on the blank, a solution from which the algae had been filtered. The tubes containing the algal suspensions and the blank were then exposed to the same source of light at equal distance and for the same length of time. Readings were made at intervals, usually of 10 minutes, on the tube containing the algae. Only initial and final readings were made on the blank. The circle readings used in the graphs represent relative amounts. For absolute values, curves must be calibrated using known amounts of the substance to be determined. Since relative values were all that were necessary in the present study this time-consuming procedure was omitted.

Experimentation

Many determinations were made but only two representative curves are presented (figs. 1, 2). Figures 1 and 2 are taken from experiments in which the readings for both formaldehyde and magnesium nitrite were made. These figures show that the reduction of nitrate to nitrite is photochemical. Where the light intensity was sufficiently low and the cultural conditions for the algae favorable, the difference between the solution containing the algae and that from which the algae had been filtered was very great. It is shown in figure 2 that at low light intensity and with the addition of phosphate, the difference in angle readings between the tube containing the algae and that containing the solution from which the algae had been filtered, was 30° at the end of the experiment. On the following day, after the tube containing the algae had been in the dark for 20 hours, it was exposed to light of higher intensity, 1.71 instead of 0.109 foot candles, along with an unexposed aliquot of the filtered solution used the day before. After the third 10-minute exposure, the reading of the blank was 54.5 as compared with a reading of 26.5 after the sixth exposure the day before. The corresponding readings for the

[1] After the preparation of this paper, checks were made by another observer, Mr. Thomas E. Booth. Since the sensitivty of his eyes was much less than that of the writer, much more concentrated algal suspensions could be used, and with more concentrated suspensions the amount of nitrite formed was always greater than that of the filtered aliquots.

[2] The minima for magnesium nitrite were used because the first preliminary work was done with algae which had grown in a magnesium sulphate solution in which the other ions were present only as impurities. The minimum or minima of any other salt of nitrous acid could be used provided the cation was always present in amounts equivalent to or greater than the nitrite.

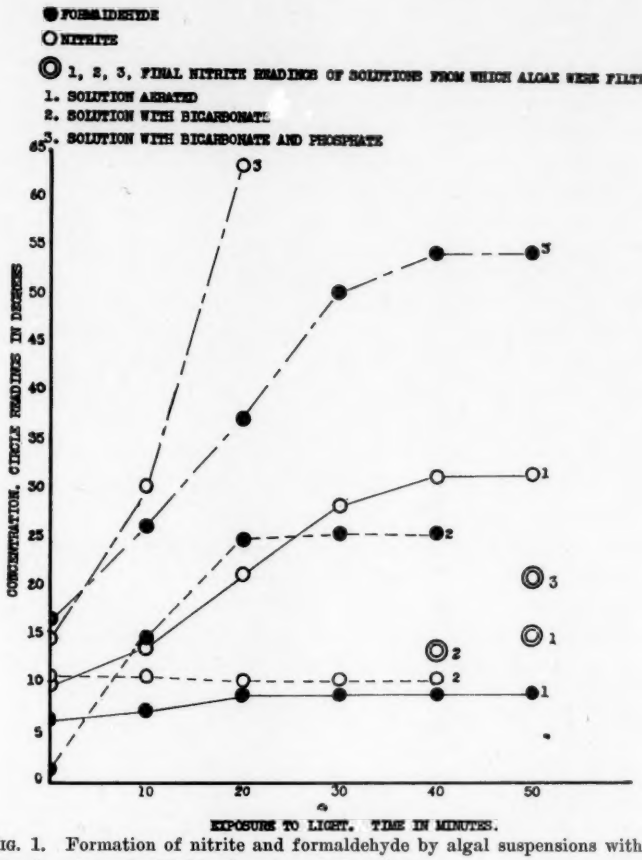

FIG. 1. Formation of nitrite and formaldehyde by algal suspensions with aeration, with bicarbonate, and with bicarbonate and phosphate.

algae were 75° (readings not taken above 75°) and 56.5°. The difference between the amount of nitrite formed by the algae and that in the solution outside of the algae is not accurately shown by the difference in angle readings because, as is shown later in the paper, there is probably a reaction taking place between the nitrite and the formaldehyde. The reading for the filtered solution indicated the total nitrite formed, while that in the tube containing the algae probably indicated only that which had not yet combined with the formaldehyde.

II. Effect of phosphate on the formation of nitrite and formaldehyde

The procedure used in studying the effect of phosphate on the formation of nitrite and formaldehyde was similar to that described in the work on

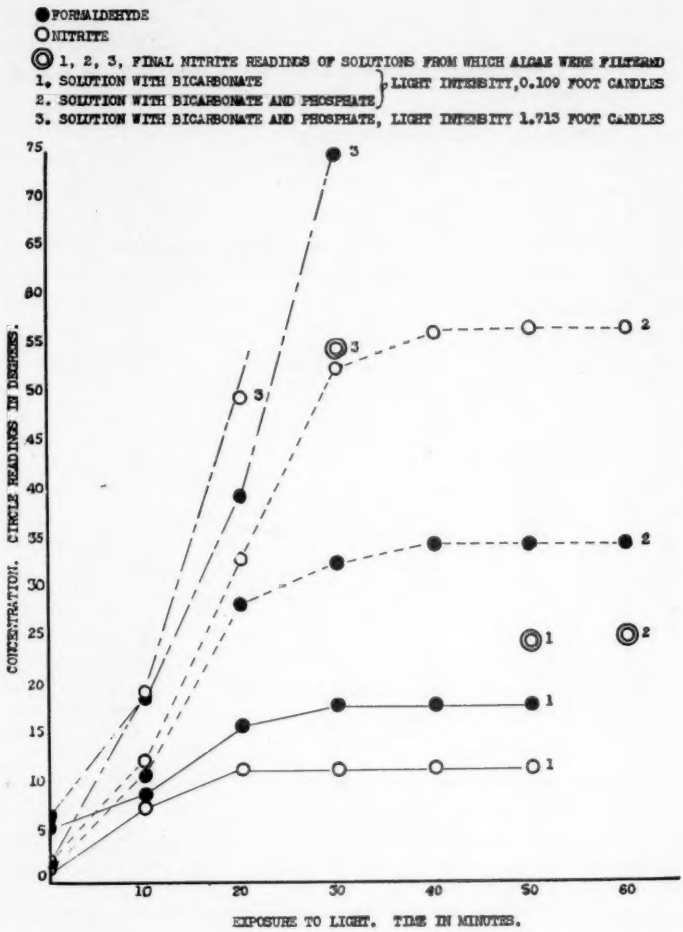
FORMALDEHYDE
NITRITE
1, 2, 3, FINAL NITRITE READINGS OF SOLUTIONS FROM WHICH ALGAE WERE FILTERED
1. SOLUTION WITH BICARBONATE
2. SOLUTION WITH BICARBONATE AND PHOSPHATE
LIGHT INTENSITY, 0.109 FOOT CANDLES
3. SOLUTION WITH BICARBONATE AND PHOSPHATE, LIGHT INTENSITY 1.715 FOOT CANDLES
CONCENTRATION. CIRCLE READINGS IN DEGREES.
EXPOSURE TO LIGHT. TIME IN MINUTES.

FIG. 2. Formation of nitrite and formaldehyde by algal suspensions, under various conditions as indicated.

nitrate reduction in the first part of this paper. The solution used for much of the work was as follows:

Salt	Per liter
KNO_3	5 mg.
$MgSO_4 \cdot 7H_2O$	10 mg.
$CaSO_4$	½ cc. saturated solution

The only phosphate present in the solution in which the algal suspensions were made was that present as impurities in the salts and gave an angle

reading of 2° which would represent an amount less than one part in 10^{11}. Two p.p.m. of acid potassium phosphate were added to study the effect of the phosphate. An amount of potassium chloride sufficient to furnish the amount of potassium added with the phosphate was added in some cases to an aliquot without phosphate, but the results were the same as when it was not added. In some cases aliquots of the same suspensions with and without phosphate were exposed at the same time, while in others a suspension was exposed the first day without phosphate and the phosphate was added after the algal suspension had been in the dark overnight and readings were again made. The results for cultures exposed the first day without phosphate and

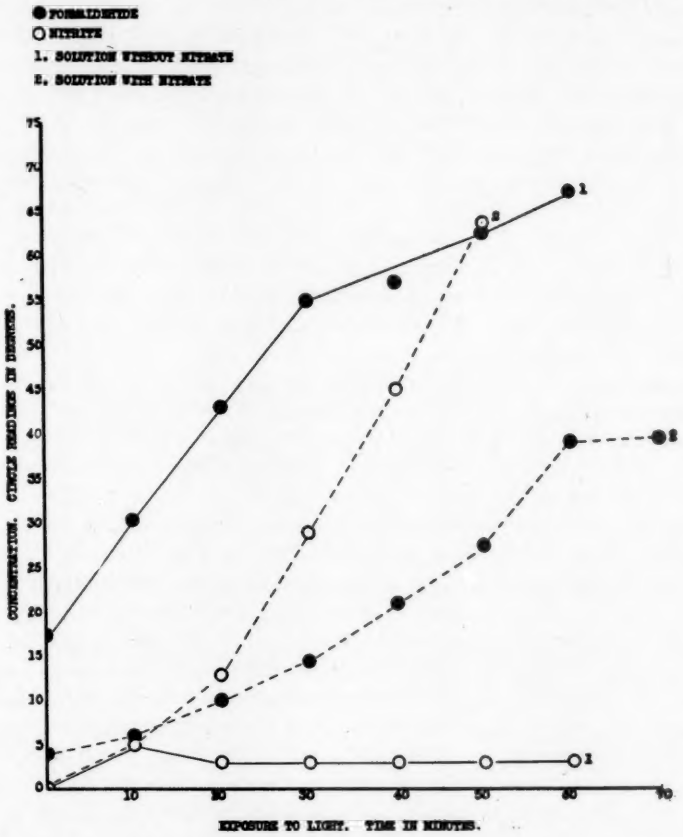

FIG. 3. Formation of nitrite and formaldehyde by algal suspensions in solutions with and without nitrate.

the following day with phosphate are shown in figures 1 and 2. It may be noted that the addition of phosphate increased the rate of formation of both nitrite and formaldehyde but that the increase was greater for the formation of the nitrite. No increase was found in the rate of nitrite formation where phosphate was added to solutions without algae.

III. Relationship between nitrite and formaldehyde concentrations within the cell

A few experiments were performed to test the theory of BALY, HEILBRON, and HUDSON (5) as to the combination of formaldehyde and nitrite in the formation of proteins. According to the theory of these investigators, activated formaldehyde combines with nitrite in the first step of protein synthesis. They say that this reaction takes place before the condensation of formaldehyde to sugars and that it is not until the amount of formaldehyde is in excess of that of the nitrite that sugars are formed.

The results obtained from experiments performed by the writer soon showed that the second part of the theory (5) was probably wrong since the amounts of nitrite indicated were lower than those of the formaldehyde only when conditions for nitrite formation were poor; that is, if no sugars were formed until the rate of formation of formaldehyde exceeded that of the nitrites, these algae could not form sugars. Furthermore, readings for dextrose (fig. 4) increased on illumination at the same time that the nitrite readings were increasing.

Experiments to show the combination of formaldehyde with the nitrites indicate that this part of the theory is correct. Figure 1 shows the results of an experiment in which the algal suspension was first aerated with carbon dioxide-free air for 5 hours in the dark and then the procedure as indicated in the first part of the paper followed. Curves 1, figure 1, show a large amount of nitrite as compared with the amount of formaldehyde. The tube was then placed in the dark until the following day. Before the initial readings for the second day, 2 p.p.m. sodium bicarbonate were added. For further evidence, algal suspensions were made in solutions in which the only nitrate present was the small amount present in the salts as an impurity. In some cases one aliquot of the suspension, as it was made up, and another to which nitrate had been added were exposed to light and observations made as described above. In other experiments the solutions were exposed to light, and readings made on the first day. After being in the dark overnight nitrate was added and light exposures were made again on the second day. Figure 3 represents the results of an experiment according to the latter method. It may be noted that when the conditions are unfavorable to formaldehyde formation, the amount of nitrite was greater than when more formaldehyde was present. On the other hand, when nitrate was absent or

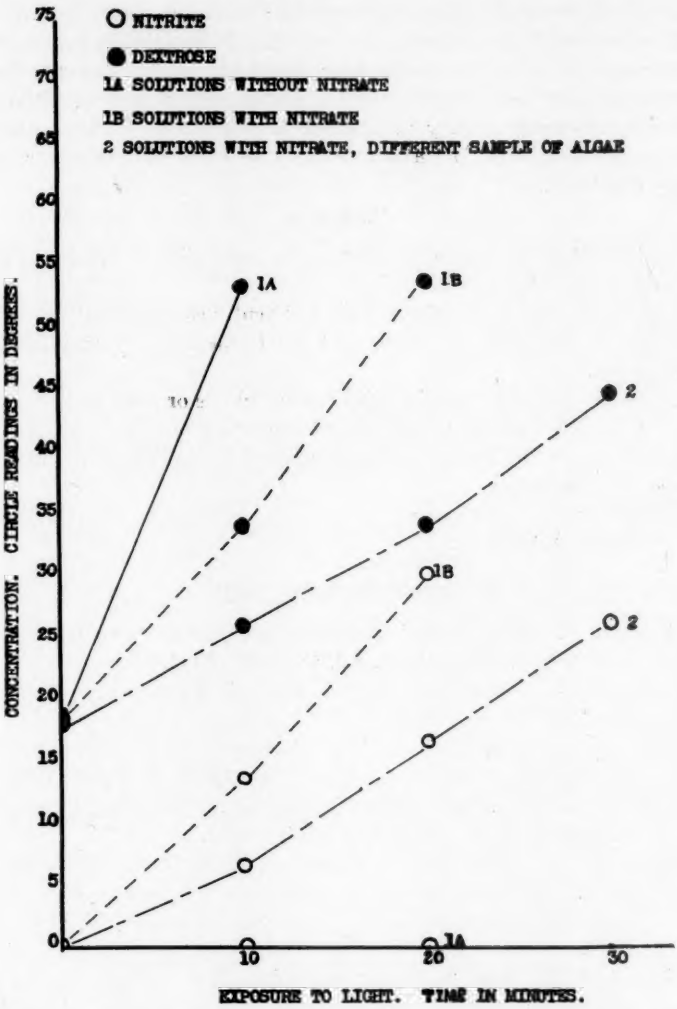

FIG. 4. Formation of nitrite and dextrose by algal suspensions in solutions with and without nitrate.

nearly absent from the solution, the formaldehyde reached higher values than when nitrate was present.

The results of these experiments are in agreement with the theory of BALY, HEILBRON, and HUDSON (5) concerning the combination of formalde-

hyde and nitrite as the first step in protein synthesis. Moreover, no trace of the ammonium ion could be detected when it was looked for in several of these studies. Considering the great sensitivity of the apparatus, about three parts in 10^{12} for most eyes and a considerably greater sensitivity for the writer, it seems unlikely that nitrite is reduced to the ammonium ion before combining with formaldehyde or some other carbon compound in protein synthesis.

Summary

1. Light plays an important part in the reduction of nitrate to nitrite within the algal cell.

2. The addition of phosphate to the nutrient medium greatly accelerates the reduction of nitrate to nitrite and the formation of formaldehyde by certain algae.

3. Nitrites probably combine with formaldehyde or some early condensation product of formaldehyde in the first step of protein synthesis.

4. Protein synthesis and the condensation of formaldehyde to sugars may proceed simultaneously.

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INFLUENCE OF THE CHLORIDE ION ON THE CONTENT OF CARBOHYDRATES IN POTATO LEAVES[1]

S. S. Baslavskaja
(WITH THREE FIGURES)

From the literature and from numerous experiments made at the Scientific Institute of Fertilizers, it is well known that high amounts of chlorides lower the percentage of starch in potato tubers. While the depressing influence of fertilizers containing chlorine on the total yield of potatoes is not always manifested, the decrease of the starch content in the tubers may be considered a constant attendant of the introduction of high amounts of chlorides.

There are some indications that the use of sulphate of potassium is preferable when fertilizing plants which are cultivated for carbohydrates (**2**). The depressing action of the chloride ion on the accumulation of starch in potato tubers may be called forth by various causes. It is known from literature (**5**) and from our experiments (**1**), that large doses of chlorides lower the content of chlorophyll in plants. Montfort (**4**), who observed a sharply decreased synthesis in leaves when immersed into chloride solutions, explains this phenomenon by the destruction and therefore by the decrease of the total amount of chlorophyll in those leaves. On the other hand the chlorides may also exert an influence on the activity of the amylase and therefore on the conversion of starch secondarily formed in the tubers. The increased conversion of starch under the influence of chlorides was observed by Schmetz (**6**), Montfort (**4**) and others. However, these authors confined themselves mostly to qualitative indices; they estimated the synthesis and the conversion of starch by the intensity of the iodine color test. Much attention has been given in this laboratory during recent years to the study of the physiological action of chlorine. It is the purpose of our work to show the influence of chlorine on the photosynthetic activity and on the accumulation of carbohydrates in the potato leaves. In this paper are presented the results of experiments concerning the influence of chlorides only on the content of carbohydrates. We considered two groups of carbohydrates, starch, and the total soluble sugars.

The experiments were conducted with an early variety of potato, "Epicure," during the years 1933 and 1934.

Experiments of 1933

In 1933 field and pot experiments were carried out. The field experiments on the Dolgoprudni Experiment Station were started according to the

[1] From the Physiological Laboratory of the Scientific Institute of Fertilizers and Insecto-Fungicides. Moscow, U. S. S. R.

following plan: Plot 1, control; fertilizers were given in the form of urea, KNO_3, and of double superphosphate; that is, without chlorine and without SO_4. Plot 2, nitrogen and potassium were given in the form of chlorides, the amount of chlorine per hectare being 195 kg.; the fertilizers were introduced the first year. Plot 3, analogous to plot 2, but the fertilizers were introduced during two consecutive years. Plot 4, nitrogen was given in the form of NH_4Cl, and potassium in the form of K_2SO_4, and the amount of chlorine was 150 kg. per hectare.

The nutritive substances were given in proportion of 60 kg. K_2O, P_2O_5, and N per hectare.

The pot experiment was started in sand cultures according to the following scheme:

Fertilizers used	Per Kg.		Per pot	
	Cl^-	$SO_4^=$	Cl^-	$SO_4^=$
NPK	0.016	0.048	0.22	0.67
" + Cl^-	0.226	.....	3.16	.....
" + Cl^-	0.436	.....	6.10	.....
" + $SO_4^=$	.....	0.336	.....	4.70

The nutritive mixture of Hellriegel was taken as a basis. The amounts of N, P, and K per pot were kept unchanged.

Planting of the field plants was carried out on May 29th; the planting in pots, on June 9th, one plant per pot.

The addition of chlorides caused a considerable decrease of the yield of tubers in the field experiment and a less abrupt decrease in tuber yield in the pot experiments.

At the time of the last harvesting on the 21st of August we had the following yields:

TABLE I

Yield data of plants of the pot experiment

Fertilizer used	Dry weight of leaves and stems	Fresh weight of tubers
	gm.	*gm.*
NPK	19.33	365
" + 3.16 gm. Cl^- per pot	18.81	382
" + 6.10 gm. Cl^- " "	19.06	342
" + 4.70 gm. $SO_4^=$ " "	18.22	365

TABLE II

FRESH WEIGHT OF THE TUBERS OF THE FIELD EXPERIMENT PER ONE PLANT

	gm.
Plot 1. Control	666
" 2. 195 kg. Cl^- one year	536
" 3. 195 kg. Cl^- two years	541
" 4. 150 kg. $Cl^- + SO_4^=$	616

No special symptoms of depression in the above-ground parts were observed in those experiments, with the exception of lighter coloring of the leaves in the plants of the series containing chlorides.

The percentage of chlorine in the plants varied according to the dose of chlorides introduced. Thus in the field experiment toward the end of vegetation, the percentage of chloride ion in the above-ground tissues of the plants of plot 1 was 0.04; of plot 2, 2.22; of plot 4, 1.94 per cent.

For analyses of carbohydrates the pot and field plants were harvested at four dates.

The plants were usually harvested at 8–9 A.M., were divided into two separate fractions (leaves, and stems) which were then carefully cut up into small pieces. An average sample was taken from the finely cut-up material and fixed in boiling alcohol. The separation of the carbohydrates into total sugars and starch was performed essentially according to the method suggested by Prof. A. KIZEL (3).

The principles of the method may be stated as follows: the soluble carbohydrates (sugars) are extracted with 82 per cent. alcohol. After evaporating the alcohol with partial vacuum at 40° C., the solution is cleared with lead acetate, and the excess lead removed with Na_2SO_4. After this the solution is hydrolyzed for 3 hours on a boiling water bath with 2 per cent. HCl.

Starch is determined in the residue from the alcohol extraction. Hydrolysis is performed by diastase. After complete saccharification (disappearance of reaction to I) the material is further filtered, and the filtrate is subjected to a 3-hr. hydrolysis with 2 per cent. HCl. In both cases the carbohydrates were determined as glucose by the method of BERTRAND.

In the field experiment triplicate and in the pot experiment duplicate samples were made.

At the last date of harvesting the field experiment, the starch was determined by the specific weight of the tubers. A marked decrease was obtained, as compared with the control plants, on the chlorine series. On the control, 14 per cent.; on the plot receiving Cl one year, 10.9 per cent.; on plot receiving $Cl + SO_4$, 11.25 per cent.

In the following tables we present only the results of the determination

of carbohydrates for only one date, as at all the other dates the results were essentially the same.

TABLE III

CONTENT OF CARBOHYDRATES IN THE LEAVES AND STEMS OF POTATOES OF THE FIELD EXPERIMENT IN PERCENTAGE OF FRESH SUBSTANCE

FERTILIZERS USED	SOLUBLE CARBOHYDRATES (SUGARS)	STARCH	TOTAL CARBOHYDRATES	RELATIVE CONTENT OF STARCH
		(a) leaves		
	%	%	%	%
NPK	0.48	0.08	0.56	14
" + Cl^- one year	0.16	0.17	0.33	51
" + Cl^- + $SO_4^=$	0.32	0.13	0.45	29
		(b) stems		
NPK	1.21	0.45	1.66	26
" + Cl^- one year	0.52	0.22	0.74	29
" + Cl^- + $SO_4^=$	0.81	0.51	1.32	38

The total of carbohydrates in the leaves, as well as in the stems of the plants of the chlorine series is considerably reduced. If we assume the total of carbohydrates of each separate series as being 100, and calculate the content of starch in relation to the latter, then there is apparent a relatively increased content of starch in the leaves and stems of the chlorine variants, which is especially sharply expressed in the leaves.

TABLE IV

CONTENT OF CARBOHYDRATES IN THE LEAVES AND STEMS OF POTATOES OF THE POT EXPERIMENT IN PERCENTAGE OF FRESH SUBSTANCE

FERTILIZERS USED	SOLUBLE CARBOHYDRATES (SUGARS)	STARCH	TOTAL CARBOHYDRATES	RELATIVE CONTENT OF STARCH
		(a) leaves		
	%	%	%	%
NPK	0.82	0.25	1.07	23
" + 0.226 gm. Cl^-	0.54	0.26	0.80	32
" + 0.436 " "	0.50	0.31	0.81	38
" + 0.336 " $SO_4^=$	0.76	0.25	1.01	24
		(b) stems		
NPK	0.82	0.22	1.04	17
" + 0.226 gm. Cl^-	0.93	0.19	1.12	17
" + 0.436 " "	0.80	0.21	1.01	20
" + 0.336 " $SO_4^=$	0.93	0.22	1.15	19

In the leaves of plants from these pot experiments one observes the same response as in the case of leaves of plants in the field experiment; that is,

with a generally smaller total of carbohydrates in the plants rich in chlorine, the relative content of starch is found to be increased. On the other hand, more or less equal figures were obtained in the case of the stems for all variants.

These data are of interest, because they offer some possibility of separating the action of K^+ from that of Cl^-. The plants of the field experiment on chlorine variants which showed an increase in chlorine content as compared with the control plants, showed also a parallel increase in content of potassium: the control, 1.7 per cent.; with a large amount of chlorine, 2.6 per cent.

On the other hand, in the pot experiments there was hardly any difference in the content of potassium between the plants of the variants NPK + 0.226 gm. Cl^-, and NPK + 0.336 gm. $SO_4^=$ per pot. In the plants of this last variant the amount of potassium was even somewhat higher. This allows us to refer the differences in the content of carbohydrates observed in the above mentioned experiments, largely to the differences in the content of chlorine.

Experiments of 1934

Only pot experiments on sand cultures according to a plan analogous to that of 1933 were carried out.

The carbohydrates were determined only in the leaves. In these experiments of 1934 it seems more interesting to represent the content of carbohydrates by curves of the daily course of their accumulation.

For analyses the leaves were cut off at definite hours: at 5 A.M., at noon, and at 6 P.M.; they were then weighed and fixed in boiling alcohol. On July 27, the material of all four variants was fixed simultaneously. On August 17, and August 21, samples from plants of two variants were taken. The method of determining the carbohydrates was the same as in the experiments of 1933.

The results are presented in table V.

Most interesting are the data of August 27 when all eight samples were taken in one day, so that all four series might be comparable with one another.

First of all, stands out the decrease of the sum of carbohydrates in the leaves of plants of the chlorine variants at noon, and in the evening. Early in the morning, however, their content of soluble carbohydrates is even somewhat greater than in the control plants. Furthermore, if the total of carbohydrates is assumed to be 100, then the relative content of soluble carbohydrates (sugar) and starch may be expressed by the following values.

The relative content of starch in the leaves of plants in the chlorine variants is higher than in plants receiving no chlorine. The excess of starch content over the soluble carbohydrate content in the chlorine variants begins

TABLE V

CONTENT OF CARBOHYDRATES IN THE LEAVES OF POTATOES IN PERCENTAGE OF FRESH SUBSTANCE

DATE	FERTILIZER USED	5:00 A.M.			NOON			6:00 P.M.		
		SUGAR	STARCH	TOTAL	SUGAR	STARCH	TOTAL	SUGAR	STARCH	TOTAL
		%	%	%	%	%	%	%	%	%
August 17	NPK	0.36	0.05	0.41	0.62	0.41	1.03	0.65	0.42	1.07
" "	" + 6.10 gm. Cl^-	0.64	0.01	0.65	0.07	0.20	0.27	0.14	0.33	0.47
August 25	NPK + 3.16 gm. Cl^-	0.08	0.01	0.09	0.28	traces	0.28	0.22	0.30	0.52
" "	" + 4.70 gm. $SO_4^=$	0.49	0.01	0.50	0.42	"	0.42	0.47	0.30	0.77
August 27	NPK	0.24	0.02	0.26	0.77	0.71	1.48	0.56	1.44	2.00
" "	" + 3.16 gm. Cl^-	0.26	0.07	0.33	0.65	0.74	1.39	0.41	1.37	1.78
" "	" + 6.10 gm. Cl^-	0.32	0.19	0.51	0.31	0.87	1.18	0.25	1.18	1.43
" "	" + 4.70 gm. $SO_4^=$	0.41	none	0.41	0.74	0.56	1.30	0.61	1.31	1.92

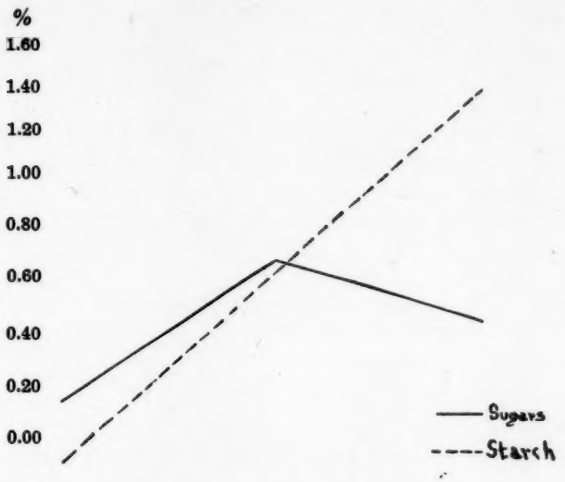

FIG. 1. Content of starch and sugars in per cent., with NPK fertilizers.

TABLE VI

RELATIVE CONTENT OF SUGAR AND STARCH IN THE LEAVES OF POTATOES ON AUGUST 27, 1934

FERTILIZERS USED	5:00 A.M.		NOON		6:00 P.M.	
	SUGAR	STARCH	SUGAR	STARCH	SUGAR	STARCH
	%	%	%	%	%	%
NPK	90	10	52	48	27	73
" +3.16 gm. Cl-	78	22	47	53	23	77
" +6.10 gm. Cl-	63	37	28	72	17	83
" +4.70 gm. $SO_4^{=}$	100	00	56	44	31	69

at earlier hours than in the control plants. This is seen especially clearly from figures 1, 2, and 3. The decrease of the total content of carbohydrates in the leaves, the relatively greater accumulation of starch in them, and the greater content of soluble carbohydrates in the morning hours in the leaves of plants of chlorine variants, all allow us to assume a slackening of the photosynthesis in those leaves, with a consequent decrease in the formation of carbohydrates, and in addition, a weaker movement of the sugars from the leaves to the roots.

Direct measurements of the energy of photosynthesis in the leaves of the potato, conducted during these experiments, confirm this assumption concerning the decreased energy of photosynthesis in the plants receiving chlorine. But these results will be reported in another communication.

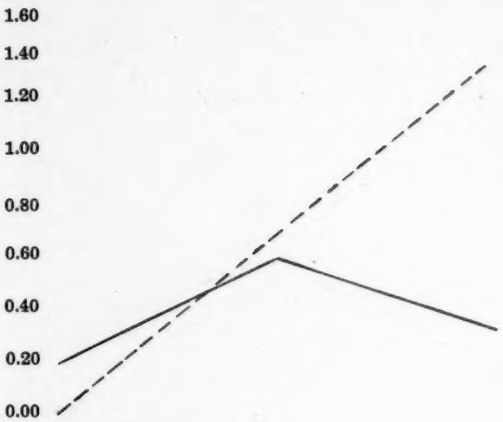

FIG. 2. Content of starch and sugars in per cent., with NPK + 3.16 gm. Cl.

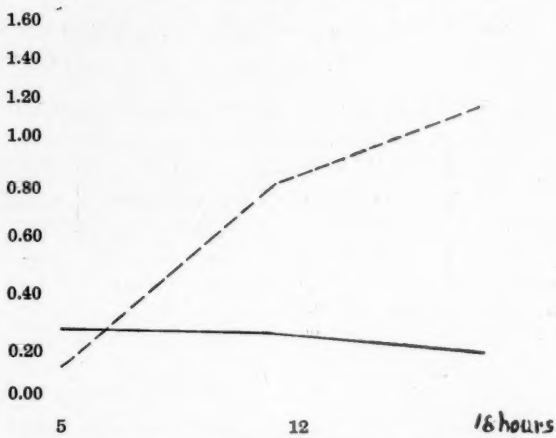

FIG. 3. Content of starch and sugars in per cent., with NPK + 6.10 gm. Cl.

The decrease of the amount of carbohydrates in the leaves of potatoes is obviously connected with the weakening of the photosynthesis activity due to a decrease in the amount of chlorophyll per unit of fresh leaf surface.

As regards the relative enrichment of the leaves with starch, we have as yet no data explaining the cause of this phenomenon. Either the movement of the starch to the tubers was retarded, or else the soluble carbohydrates were utilized more energetically in the respiratory process.

Studies of the influence of chlorine on the formation and hydrolysis of

starch, and on the activity of the enzymes concerned in carbohydrate metabolism are being carried out in our laboratory.

The general conclusions from the work presented in this paper are as follows:

1. Large doses of chlorides decrease the total sum of the carbohydrates in the leaves. This is obviously connected with a lowered content of chlorophyll per unit of leaf surface and a weakened photosynthetic activity.

2. The leaves of plants of the chlorine series are relatively richer in starch than the leaves of the control plants. Further investigations are necessary to explain this phenomenon.

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HYDRATION STUDIES IN FRESH AND DRIED RED CLOVER ROOTS AND SHOOTS WITH REFERENCE TO PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION OF TISSUE

GLENN A. GREATHOUSE AND NEIL W. STUART
(WITH THREE FIGURES)

Introduction

It is well known that plant tissues which have been thoroughly dried readily absorb atmospheric moisture. This process has been termed rehydration.

In recent years it has been established that living plant tissues bind a portion of the water associated with their cell constituents. It is considered that this bound water possesses properties differing from those of the remaining water in the cells. It has been proposed that this water-binding capacity of plant tissues is of importance in such psysiological phenomena as cold resistance, drought hardiness, and growth. The writers (**4, 5**) have reported that the unfreezable or bound water content of the tissues effectually differentiated several varieties of red clover which exhibited varying degrees of cold hardiness.

The purpose of the present investigation was to compare the unfreezable water content of the fresh clover roots and shoots with the rehydration of the dry tissue. Detailed analyses were performed on the tissue thereby permitting a study of the correlation existing between composition of the tissue and its unfreezable water content of the fresh tissue or rehydration capacity of the dried tissue.

LITERATURE REVIEW

ROSA (**9**) states ". . . . . the greater water-retaining power of the hardy tissue (cabbage) evidently does not depend entirely on the organization of living matter, but on the chemical and physical properties of the substance of which the tissues are composed." STRAUSBAUGH (**11**) thought that the capacity of plant organs to take up or imbibe large amounts of water must not necessarily be taken as an index of their power to retain water when exposed to conditions favoring undue water loss such as freezing or drying. HOOKER (**6**) showed that the hardier parts of apple shoots, the bases, have a greater water-retaining power than the tips, which are less cold-resistant. He placed portions of air-dried ground tissues in desiccators over H_2SO_4, the concentration of which ranged from concentrated to 36.69 per cent. HOOKER attributed the greater water-retaining power of the hardy tissue

to the larger percentage of total pentosans found therein. SHREVE (**10**) determined the rate of evaporation from the tissue of *Encelia farinosa*, using dry discs of the plant tissue in the determinations of imbibitional capacity. She found that the rate of evaporation or dehydration varied inversely with the imbibitional capacity and directly with the original water content. BOSWELL (**1**), working with cabbage and tomato leaves from plants of known degrees of hardiness, showed that the hardy tissue lost water less rapidly than the tender tissue. He concluded that the comparative rates of water loss, under uniform drying conditions, were a measure of the relative hardiness of certain kinds of plant tissue. CHRYSLER (**2**) noted that the amount of imbibed water bound per gram of dry kelp increased with an increase in hydration, but not proportionally. Her data indicate that the proportion of dry material in a colloidal system is a factor which greatly affects the weight of water bound per gram of dry material. CRIST (**3**) states that the lack of close and consistent correlation between individual nutrients, bound water, hydration capacity, total water content, water requirements, and rate of dehydration of the same plants indicates that other factors are of great importance in influencing certain features of the plant's behavior. Colloidal gels made to simulate the normal plant tissue did not conform closely to the behavior of the tissue in dehydration.

Recently, MEGEE (**8**) found no direct relationship between winter hardiness in alfalfas and heat of wetting, swelling, moisture equivalent, and amount and rate of loss of moisture in roots of hardy and non-hardy plants. A number of his methods have been used in the study of soils and cannot be applied accurately to plant tissue.

Materials and methods

The data from the physical and chemical measurements reported by the writers (**5**) elsewhere have been plotted so that they may be compared with the rehydration curves on oven-dried portions of the same root and shoot tissues. Portions of the clover roots and shoots obtained at each sampling period were dried at 60° C., and ground so as to pass through a 100-mesh sieve. Duplicate samples of approximately 1 gm. each were placed in weighing bottles and dried to constant weight at 80° C. and 3 to 4 cm. pressure. All of the samples were placed in a large desiccator over H_2SO_4 having a specific gravity of 1.2281. This concentration of acid was calculated to produce a relative humidity of 75 per cent at 30° C. The desiccator containing the samples was placed in an air thermostat controlled at 30 ± .3° C. Care was used so that the pressure in the desiccator was equal to that of its surroundings. The samples remained under these constant conditions until they had reached equilibrium as determined by their constant weight. Moisture equilibrium was reached in approximately 3 weeks.

Experimental results

The results are presented graphically in figures 1, 2, and 3. Figure 1 shows that the hydration capacity (unfreezable water) in the fresh root tissue was higher in the Ohio (hardy) variety at all the sampling dates except those in September. A similar relation was shown for the rehydration values of the dried tissue. The hydration capacity of the fresh shoot tissue in the Ohio and French varieties was similar to that of the root tissue, except that the values were of a lower magnitude. The rehydration capacity of the shoot tissue of both varieties was higher than that of the roots at all the sampling periods, except the period in January. The greatest deviation in hydration capacity in both Ohio and French roots and shoots occurred in January.

It can be noted from figure 1 that the total pectin in the roots and shoots of the French (less hardy) variety was frequently greater than that of the

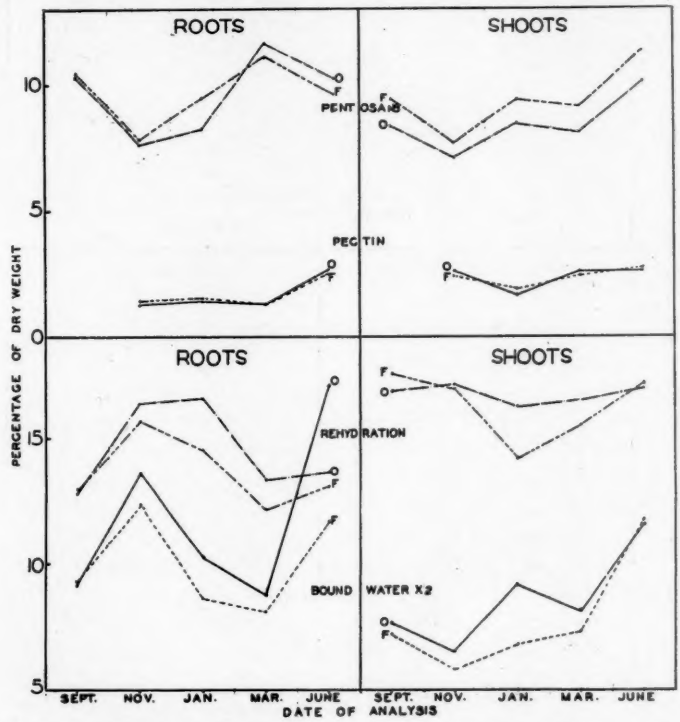

FIG. 1. Bound water (percentage of the total), rehydration, pentosans, and pectin in clover roots and shoots.

Ohio variety. Total pectin does not seem to be related to the greater hydration capacity of the hardier clovers.

The pentosan content (fig. 1) of the roots was inversely related to the hydration capacity of the fresh and dried clover tissue. The pentosan content and the hydration values of the shoots gave similarly shaped curves; yet the pentosan content of the tissue cannot be responsible for the greater hydration capacity, since the hardier variety has the smaller percentage of pentosans.

Figure 2 shows the percentage of starch and dextrins, sucrose, protein, and non-protein nitrogen in the roots and shoots of the Ohio and French red clover.

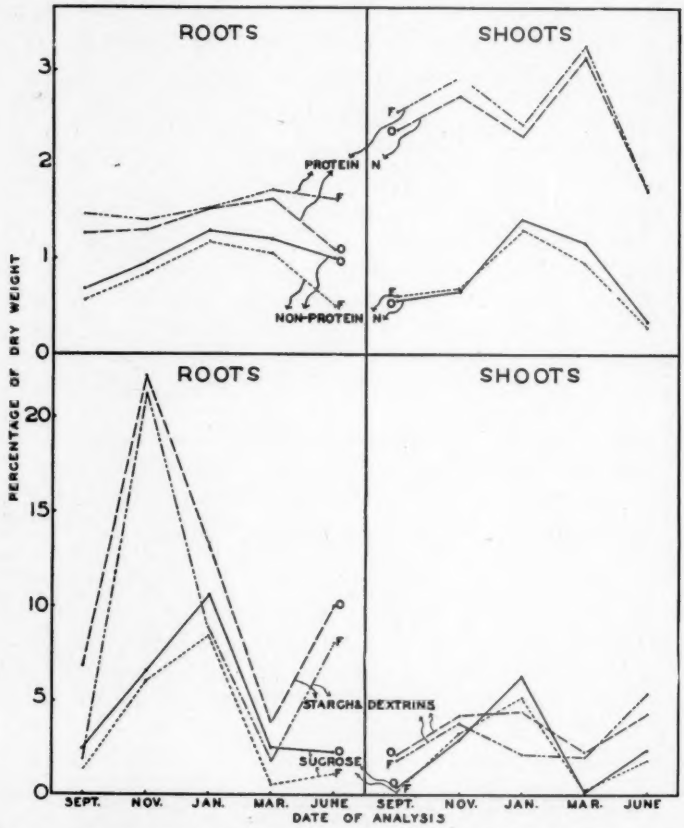

FIG. 2. Sucrose, starch and dextrins, protein nitrogen, and non-protein nitrogen in clover roots and shoots.

The starch and dextrin content of the roots seems to be related to the hydration capacity of this tissue. The sucrose (a hydrated sugar) does not seem to be directly related to the hydration capacity of the root tissue. If the starch, dextrin, and sucrose percentages were added together and plotted, the resultant curve would follow the hydration capacity of the root tissue. This tends to smooth out the starch, dextrin, and sucrose curves (fig. 2) and makes them more closely coincide with the hydration curve. The same relationship does not hold for the shoot tissue.

The percentage of proteins (fig. 2) in the roots and shoots of the French red clover was always higher than in the Ohio. Furthermore, the proteins were inversely related to the hydration capacity of both roots and shoots. The curves of the non-protein nitrogen for the roots and shoots do not follow the hydration capacity curves, especially is this evident in June when an inverse relation exists.

Figure 3 shows the osmotic pressure and total water fluctuations in roots and shoots.

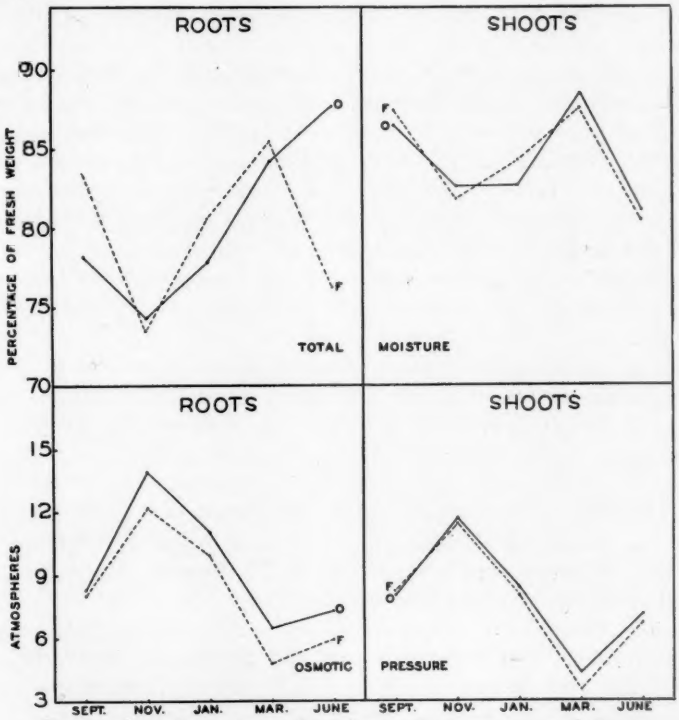

FIG. 3. Osmotic pressure and total water in clover roots and shoots.

The curve of the osmotic pressure for the roots follows in general the hydration capacity of the tissue. However, this relationship does not hold for the shoots. No consistent relation exists between total water content of the roots and shoots and the hydration measurements.

Discussion

The hydration capacity of red clover tissue effectively differentiated varieties which exhibited varying degrees of cold hardiness. Therefore, it is of prime importance to know what factors influence this capacity. In order that these influences may be noted the data of certain physical and chemical measurements were graphed (figs. 1–3).

Figures 1, 2, and 3 show that the same factors do not equally influence the hydration capacity of the roots and shoots. This means that in order to obtain a correct concept of cold hardiness both the root and shoot should be analyzed. Just why the unfreezable water fraction is of greater magnitude in the root than in the shoot, whereas the rehydration values are larger in the shoot, with the exception of the January analysis, is difficult to explain.

Rosa (**9**), Hooker (**6**), and other investigators at the Missouri Agricultural Experiment Station have proposed that pectin and pentosans are related to cold hardiness. Many of these investigations were made on a single variety, using only the shoot tissue. The present investigation shows that if only one variety of red clover had been studied the hydration capacity (unfreezable water) and the pentosan content would appear to have been correlated in the shoots but not in the roots. However, since two varieties were studied and it has been shown that the variety with the higher pentosan content has the lower hydration capacity no relationship can be obtained.

The hydration capacity of the tissue appears to be related to the total carbohydrate content, especially the sugar, starch, and dextrins (figs. 1, 2). It is difficult to evaluate any one of the carbohydrate fractions with respect to its influence on the hydration capacity of the tissue. This is to be expected since the hydration capacity is the resultant of all the osmotic and imbibitional forces present within the tissue. Sucrose is a hydrated sugar and would influence the water equilibria in tissues differently than dextrose. However, the influence of the precentage of sucrose on the hydration capacity differs in the root and shoot.

Neither the protein nitrogen nor the non-protein nitrogen appears markedly to influence the hydration capacity of the tissues. MacDougal (**7**) showed that very dilute solutions of amino acids favor imbibition in artificially prepared biocolloids.

The positive correlation between rehydration of the dried tissue and the unfreezable water of the fresh tissue shows that the hydration capacity does not depend entirely on the organization of living matter, but to a certain extent on the chemical composition of the tissues. Therefore the degree of cold hardiness in the clover plant should be modified by environmental factors which tend toward greater accumulation of food reserves. Experiments are in progress to test this hypothesis.

Summary and conclusions

1. The Ohio and French varieties of red clover can be differentiated as to cold hardiness by the unfreezable water of the fresh tissue or the rehydration of the dried tissue.

2. It is difficult to evaluate the internal factors which influence the hydration capacity of a given tissue. These factors appear to be different for root and shoot tissues. The readily available carbohydrates (sugars, starch, and dextrins) seem to be the most important in favoring a greater hydration capacity. The total pectin and total pentosans were not related to the hydration capacity of the red clover root and shoot tissues. The protein nitrogen was usually higher in the tissue having the lower hydration capacity. Similarly, the non-protein nitrogen curves did not follow the hydration capacity curves.

3. No consistent relation appears between the total water content of the roots and shoots and the hydration measurements.

4. The hydration capacity of red clover tissue is influenced by the chemical composition as well as by the organization of living matter.

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BRIEF PAPERS

A SIMPLE GAS ANALYSIS APPARATUS FOR THE MEASUREMENT OF PLANT RESPIRATION

(WITH TWO FIGURES)

For the quantitative measurement of the respiratory gaseous exchange in plants various forms of gas analysis apparatus have been used. As examples of those most commonly employed for the purpose may be mentioned the BONNIER and MANGIN apparatus and the HALDANE gas analysis apparatus. In the BONNIER and MANGIN apparatus the operations of sampling and cleaning are rather troublesome. Drying the measuring tube after each analysis consumes time, and errors are likely to be introduced if the subsequent analyses are carried out in a wet tube. The HALDANE gas analysis apparatus, however, possesses a high degree of accuracy but requires considerable experience before it can be used with discrimination. Moreover, the construction of the apparatus is complex and an accidental breakage in the glass parts is not easily repaired.

In the course of investigations on the gas storage of fruits the need was felt for a simple and effective means of gas analysis. With this object in view a simple apparatus was constructed in this laboratory and has been in use for some time with satisfactory results.

The principle of the apparatus consists, in brief, in measuring the pressures exercised by the various constituents of a gaseous mixture. As the partial pressure of a component is proportional to its concentration in the gaseous sample and the sum of the various partial pressures is equal to the total pressure exerted by the gas sample, the percentage content of the component, say x, is easily calculated:

$$x = h \times 100/H_o.$$

where h = the partial pressure of the component under consideration,
and H_o = atmospheric pressure in mm. Hg.

The apparatus (fig. 1) consists essentially of a manometer (made out of a 3 mm. bore glass tubing), one limb of which has a 200-mm. metal-enamelled scale attached to it. The "0" mark on the scale corresponds with a circular mark (X) on the burette (B), the volume of which between the stopcock (G) and the circular mark (X) is 5 cc. The manometer tube and the burette (B) are inclosed within a glass jacket the water in which is kept stirred by means of an air-blower (A). A leveling bulb (L) is provided with which sampling is done and the mercury level in the manometer adjusted. The two bulbs (C) and (D) are similar to the "combustion pipette" employed by HALDANE

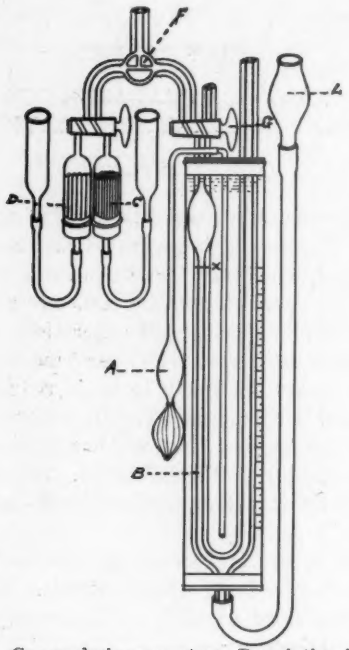

FIG. 1. Gas analysis apparatus. Description in text.

(**2**), except that the ignition tubes inside the pipette have been removed. One bulb is filled with fine glass tubings of suitable length and contains a 30 per cent. solution of KOH so that 5 cc. of air can be introduced into it, whereas the other contains stick yellow phosphorus which is kept under water. The use of phosphorus (**1**) instead of potassium pyrogallate as an absorbent for oxygen obviates the necessity of keeping the gaseous sample in a state of continuous agitation, which is often very tiring. The phosphorus bulb is kept covered from the light by means of a metal shield which is taken off only during analysis.

The air in the apparatus is first freed from CO_2 and oxygen in order that all of the capillaries may be filled with nitrogen. Sampling is done by the washing method (**1**), by interposing a 3-way stopcock between the inlet of the burette and the respiration chamber. The gas samples are drawn in and sent out by means of the mercury leveling bulb, the number of washings depending upon the amount of gas available. As a rule, two or three washings give satisfactory results. After the final washing is completed, exactly 5 cc. of the sample is taken into the burette, the mercury being adjusted to the mark (X) by means of the leveling bulb. The subsequent process consists

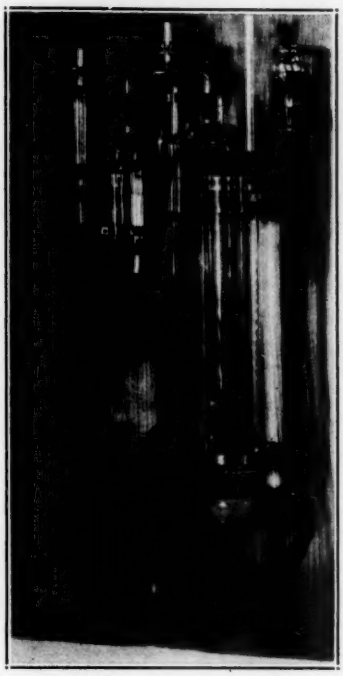

FIG. 2. Photograph of the apparatus mounted for use.

in sending the gaseous sample back and forth several times into the KOH bulb. When the CO_2 has been completely absorbed the gas is finally brought back into the burette, the potash level is set, and the stopcock (*G*) half turned. It will now be found that the gas has shrunk in volume. By means of the leveling bulb the mercury is again brought to the mark (*X*) and the lowering of pressure is read. The oxygen is absorbed by a similar process except that the gaseous sample need not be continuously agitated. Through the stopcock (*F*) the pressures in the absorption bulbs are equilibrated with atmospheric pressure. The lowering of pressure after each absorption represents the partial pressure of the component absorbed from which the percentage content of the constituent is easily computed.—B. N. SINGH and P. B. MATHUR, *Benares Hindu University, India.*

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NOTES

Thirteenth Annual Meeting.—The American Society of Plant Physiologists will meet in its thirteenth annual session at Atlantic City, New Jersey, December 29, 1936. As usual, the meetings will extend through several days. The arrangements as set forth here are preliminary, and members should verify the announcements from information to be published later by the A.A.A.S. in *Science*. The headquarters of the Society will be at the Ambassador Hotel, and the scientific sessions will probably be held in the Auditorium. The usual joint meetings with the American Society for Horticultural Science, the Physiological Section of the Botanical Society of America, and Section G, A.A.A.S., may be expected. The annual dinner is scheduled for Tuesday evening, December 29. It is customary to announce the awards of honors at the close of the annual dinner. This year the fifth award of the STEPHEN HALES prize, and the twelfth award of the CHARLES REID BARNES life membership are to be a part of the after-dinner program. No one will want to miss these festivities. Tickets for the annual dinner should be purchased as soon as possible after arrival in order that the secretary may make suitable reservations for every one. The officers of the Society will welcome and appreciate the cooperation of the members in all matters pertaining to the successful conduct of the meetings.

Committee on the Stephen Hales Award.—The personnel of the STEPHEN HALES award committee is defined in the constitution and by-laws of the Society. The committee on the fifth award is constituted as follows: Dr. W. W. GARNER, U. S. Department of Agriculture, chairman; Dr. HUBERT BRADFORD VICKERY, Connecticut Agricultural Experiment Station; and Dr. CHARLES A. SHULL, the University of Chicago. Each member of the committee serves for six years, and by rotation, becomes chairman during the last two years of his tenure of membership. The announcement of the award will be made at the annual dinner of the Society.

Committee on the Charles Reid Barnes Life Membership.—The committee chosen to select the recipient of the twelfth award of the CHARLES REID BARNES life membership in the American Society of Plant Physiologists has been appointed by President R. B. HARVEY as follows: J. W. SHIVE, Rutgers University, chairman; Dr. H. A. SPOEHR, Carnegie Institution of Washington; Dr. SAM F. TRELEASE, Columbia University; Dr. EDWIN C. MILLER, Kansas State Agricultural College; and Dr. E. S. JOHNSTON, the Smithsonian Institution.

Reissue of Early Volumes of Plant Physiology.—The executive committee of the Society has been considering the feasibility of issuing a reproduction of volumes 1 and 2 of PLANT PHYSIOLOGY, which have been unavailable for a number of years. In order to determine whether there is sufficient demand to pay the costs of reproducing these volumes, members who would be willing to pay the usual price to obtain them are requested to write to the secretary to that effect. It would aid also if members would examine the files of PLANT PHYSIOLOGY in their respective institutional libraries, and if possible secure a pledge of subscriptions if the library does not possess these early volumes. If sufficient interest is shown, photographic reproduction of the first two volumes could be financed without difficulty. The secretary and other officers would be pleased to know whether there is interest enough to warrant the expenditure of funds for this purpose.

Errata.—The attention of the editors of PLANT PHYSIOLOGY has been called to a number of errors in the current volume. The list is not complete, but includes only those which have been reported by authors. This list of errata is to be found at the close of the table of contents of volume 11. It is a useful custom to enter these corrections at the points where the errors occur. This is especially desirable in connection with tabulated data, arabic numerals, decimal points, etc., errors which are difficult to detect in casual reading.

We are indebted to Dr. OTIS F. CURTIS for several corrections to the key list of names for identification of plant physiologists in attendance at the Sixth International Botanical Congress, which accompanied the reproduction of the photograph in the January 1936 number of PLANT PHYSIOLOGY. Numbers 113 and 114 are transposed in the key. No. 113 is K. V. THIMANN, and no. 114, R. EMERSON. In addition, no. 121, left unidentified in the key, is W. SCHUMACHER, whose name was recorded as no. 115. This leaves no. 115 unidentified. If any other errors are noted in this key, we hope corrections will be sent to us for publication.

Manuscripts.—Authors are urged to make their papers as brief and clear as possible. With many investigators engaged in similar lines of work, a general reviewing of the past literature by each author is unnecessary. There is too much repetition, too much waste of space and funds in the reproduction of long literature lists with identical citations. By eliminating this reappraisal of the entire background of research for a time, presenting just that which is new, with its immediate bearing upon other recent work, authors can guarantee a more prompt publication of their papers.

In other ways authors can contribute to more rapid handling of their papers. Legends, for instance, should not be attached to the photographs

or drawings. The figures go to the engravers, and the legends to the type-setters. They should therefore be separate. The figures should be numbered properly on the back of the copies sent, and bear the author's name: the legends corresponding, properly numbered, should be type-written on a separate sheet as part of the manuscript.

Frequently literature citations are sent in incomplete form, or not in alphabetic order. Papers containing defective citations, or literature lists otherwise lacking in conformity to the style used in PLANT PHYSIOLOGY, are automatically delayed in being sent to the printer. They must wait until the editor can find time to return the list to the author, or until the citations can be amended by editorial service. This sometimes requires much time because of the pressure of other duties. Early publication is facilitated by attention to such details as are mentioned.

The Living Garden.—An unusually interesting and valuable garden book has been written by Dr. E. J. SALISBURY, Quain Professor of Botany in University College, London. The author presents garden plants as living organisms, responding to the conditions of their environment. The story is told with a minimum of technical terms, yet one realizes as he reads that he is being led by a master into the fascinating knowledge of how plants live, grow, and reproduce their kind; how they compete with their neighboring plants; how they respond and adapt themselves to their changing environment. The book contains seventeen chapters replete with information that could be provided only by one who knows and loves plants, and who has watched them grow through many seasons. Every young botanist, and especially those interested in the behavior of plants, should read it; for it has a style and quality that inspires to deeper study. The publishers, the Macmillan Co., New York, offer it to the public at $3.00 per copy.

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